

Optical coherence elastography

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Abstract

Optical coherence elastography (OCE) is a non-invasive imaging technique for the mechanical characterization of biological samples in three dimensions at a variety of length scales – from tissues to cells – and that is undergoing rapid development. OCE uses optical coherence tomography to measure the deformation of the sample induced by mechanical loading. A suitable model is applied to estimate a mechanical parameter, such as Young's modulus, which is then mapped into an image. Over the past two decades, several key features of OCE have considerably developed, including micro-scale spatial resolution and high imaging speed, making OCE viable for biomedical applications, particularly in ophthalmology and oncology. In this Primer, we provide a comprehensive description of OCE. We begin with an overview of the working principles of OCE and its variety of implementations, including experimental set-ups and signal processing methods. We then detail techniques used to induce and measure deformation in OCE and describe prominent OCE application areas, such as the characterization of tissues, cells and biomaterials. Finally, we highlight several key advantages and limitations of OCE and we provide perspectives on likely future advances and opportunities in OCE.

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Introduction

The mechanical properties of tissues and cells are fundamental to physiological function¹. Importantly, disease modifies tissue mechanical properties, providing a biomarker for disease detection. Hence, manual palpation has been, and remains, a prominent diagnostic tool in many areas of clinical medicine².

Inspired by manual palpation, the past 30 years have seen the sustained development of elastography³, a technique consisting of the creation of maps of a tissue's mechanical properties, termed elastograms. An elastogram is obtained by applying a mechanical load on the sample and measuring the resulting deformation. Solid mechanics is used to estimate mechanical properties of the sample from its deformation – most commonly shear or Young's modulus – to create elastograms. The most prominent developments have been in clinical elastography, where either ultrasound⁴ or magnetic resonance⁵ imaging is used to map tissue deformation at a spatial resolution on the scale of millimetres and to imaging depths of centimetres³. These techniques are now routinely used in many clinical applications, including the diagnosis of breast cancer⁶ and staging liver disease⁷.

Altered mechanical environments at the cellular scale have been linked with the onset of many pathologies⁸, including cancer⁹ and cardiovascular disease¹⁰. The mechanical properties of subcellular structures, including organelles and molecules, are measured with nanoscale methods, such as atomic force microscopy (AFM)¹¹ and optical tweezers¹². These techniques are contributing to the development of mechanotherapies that aim to treat disease by modifying mechanical properties to correct abnormal cellular functions¹³. However, these techniques are performed point-by-point, resulting in extended imaging times, and are inherently limited to two-dimensional (2D) characterization.

Optical elastography techniques¹⁴ have been developed to map the mechanical properties of tissue on the mesoscale, that is, between that of cells and organs (~1 μm to ~1 mm). Importantly, disease often presents clinically at this scale, suggesting clear potential to improve diagnostics. In addition, optical elastography may contribute to the clinical translation of discoveries in mechanobiology by reconciling tissue mechanics from the cellular level to the whole-organ level. Optical elastography has been developed using a range of techniques, including optical coherence tomography (OCT)^{15,16}, Brillouin microscopy¹⁷, laser speckle imaging¹⁸, full-field digital holography¹⁹ and ultrafast microscopy-based elastography²⁰. In this Primer, we focus on OCT-based elastography, that is, optical coherence elastography (OCE). An advantage of using OCT^{21,22} is that 2D and 3D maps of tissue displacement can readily be acquired in timescales of milliseconds and seconds, respectively. Furthermore, OCT can provide a spatial resolution of ~5–30 μm with imaging depths of ~1–2 mm in highly scattering tissues such as skin²². The use of phase-sensitive detection²³ allows the spatial resolution of local sample displacement measurements to be pushed to nano- and even subnanometre in tissues²⁴.

In OCE, the characteristics of the mechanical load and the resulting spatiotemporal distribution of sample deformation determine the appropriate solid mechanics equations to use for estimating mechanical properties^{3,25}. A main challenge is that biological tissues are mechanically heterogeneous and exhibit both elastic (time-independent) and viscoelastic (time-dependent) properties¹. Furthermore, tissues are often poroelastic, that is, they comprise liquid as well as solid phases. A thorough characterization of a tissue's mechanical properties would hence require prohibitively complex

and time-consuming measurements and accompanying numerical methods that would probably render OCE infeasible for most biomedical applications. For this reason, very stringent assumptions on the nature of tissue deformation are often imposed to enable straightforward solutions to the inverse problem of elasticity, that is, estimating elasticity. This most commonly leads to obtaining the sample shear modulus or Young's modulus from measured deformations^{3,15,16,25}.

The two most prominent OCE techniques are compression OCE¹⁵ and transverse wave OCE¹⁶. In compression OCE, a quasi-static step or ramp load is applied to the sample, and stress and strain – described by the tensors σ and ϵ , respectively – can be calculated from the measured deformation, allowing Young's modulus to be estimated from Hooke's law^{1,3,25}. In transverse wave OCE, localized transient loading is applied to the sample, which induces laterally propagating mechanical waves. The measured wave velocity is related to the shear modulus using appropriate mechanical wave models²⁵. Several other OCE techniques have also been proposed to measure time-dependent axial deformation (parallel to the imaging beam). Here, we classify these techniques as axial wave OCE, which includes axially transient OCE²⁶, harmonic OCE²⁷, and vibrational OCE²⁸.

OCE has been applied in a range of clinical settings and, similar to OCT²², heavily in ophthalmology²⁹. In this field, non-contact techniques, such as transverse wave OCE or harmonic OCE, have been developed to map the elasticity of the anterior segment of the eye to detect and monitor pathologies such as keratoconus^{30–32}. OCE has also undergone extensive development as a tool for intraoperative identification of tumour margins^{33,34}, most prominently in breast-conserving surgery³⁵. The relatively high imaging speed and high spatial resolution of OCE^{36–39}, together with the potential for integration in compact imaging probes^{40–42}, makes it an attractive solution to this large unmet clinical need. OCE has also been proposed in areas such as cardiology^{43–47}, dermatology^{48–53} and pulmonology^{54,55}, where there is an established link between variations in micro-scale mechanical properties and disease progression. These OCE application areas are relatively immature at this time owing to the need for relatively complex and specialized OCE probes, such as endoscopes and handheld probes, which are only now approaching maturity for extensive clinical studies^{42,56}. In mechanobiology, high-resolution OCE has been demonstrated to map cellular and intercellular elasticity in 3D, both in single cells^{57–59} and cell spheroids^{60,61}. The capability to map 3D elasticity on the cellular scale to depths of hundreds of micrometres enables the study of cells in their native environment, which is more challenging with other established techniques, such as AFM, which is limited to 2D surface measurements, or optical trapping⁶² and micropipette aspiration⁶³, which are limited to studying cells in isolation.

In this Primer, we present the main approaches used to measure sample deformation in OCE, that is, phase-sensitive detection and correlation-based methods. We then present the principles behind the three main groups of OCE techniques, namely, compression¹⁵, transverse wave¹⁶ and axial wave OCE. In each case, we describe the mechanical loading paradigms and the mechanical models used to relate OCE measurements to mechanical parameters. In the 'Results' section, we detail the data acquisition protocols and signal processing methods used to generate elastograms in the two most prominent OCE methods: compression¹⁵ and transverse wave OCE¹⁶. Then, in the 'Applications' section, we provide an overview of the main areas in which this technique is used, with particular emphasis on oncology, ophthalmology,

cardiology, dermatology and mechanobiology. We then detail the technical characterizations of OCE, describe its capabilities and developments giving an overview of how emerging research is pushing the boundaries of the technique and, finally, we discuss the exciting prospects for OCE.

Experimentation

Here, we briefly describe OCT^{21,22} to then discuss the main methods used to measure deformation in OCE and the techniques to estimate elasticity from OCE measurements.

OCT

OCT is a non-invasive optical imaging technique providing micrometre-scale spatial resolution to penetration depths of 1–2 mm in optically scattering tissues, for example, skin or breast tissue^{21,22}, which alter the direction of incident light due to intrinsic inhomogeneities in refractive index. Depth-resolved imaging is achieved using low coherence interferometry, where contrast is based on the optical backscattering of the tissue itself without the need for any exogenous contrast agent. Fourier domain OCT systems are the most widely used, where the spectral interference pattern is detected in the frequency domain and the complex OCT signal ($I(z)$) is retrieved with a Fourier transform. The obtained signal can be defined in one dimension along the tissue depth (z) obtaining a so-called A-scan. This can be written as^{22,23}

$$I(z) = A(z)e^{i\varphi(z)} \quad (1)$$

where A is amplitude and φ is the phase of the A-scan. The intensity of this complex signal ($|I(z)|^2$) is the OCT structural image. In most contemporary OCE techniques, displacement is measured from the phase in equation (1). Fourier domain OCT systems are divided into spectral domain and swept source OCT²², and both are used in OCE^{15,16,64}. Spectral domain systems are often preferred for their intrinsic displacement stability and straightforward implementation^{16,64}. Swept source systems are generally more complex and costly, but also have attractive features for OCE, such as higher imaging speed, longer axial imaging range and penetration depth²², and they could potentially achieve greater displacement stability than spectral domain OCT with proper synchronization^{65,66}.

In OCT, the axial and lateral resolution are largely decoupled²². The axial resolution is determined by the mean wavelength of the source (λ_0), which is often the central wavelength, and its bandwidth ($\Delta\lambda$), defined as the full width at half maximum of its wavelength spectrum. For a Gaussian shaped source spectrum, the axial resolution (δ_z) in OCT is^{21,22}

$$\delta_z = \frac{2 \ln 2}{\pi n} \frac{\lambda_0^2}{\Delta\lambda}, \quad (2)$$

where n is the refractive index of the sample, often assumed to be 1.3–1.4 in soft tissues. The lateral resolution (δ_x) is determined by the characteristics of the objective lens in the sample arm of the OCT system and the incident beam size and is given by²²

$$\delta_x = \frac{4\lambda_0 f}{\pi d} \quad (3)$$

where f is the focal length of the objective lens and d is the size of the incident beam on the objective lens.

Several methods have been developed to measure tissue deformation from the complex signal (equation (1)). In early OCE studies⁶⁷, for example, full-field OCT and time-domain OCT systems, where the complex signal $I(z)$ is not easily accessible by a simple Fourier transform as in typical Fourier domain OCT, tissue deformation is obtained from the signal intensity via correlation-based approaches. The majority of current OCE techniques^{17,18} instead give access to the whole complex signal after Fourier transform and allow retrieval of tissue deformation with phase-sensitive approaches. Subsequently, existing methods can broadly be grouped as phase sensitive or correlation based.

Phase-sensitive detection. The phase (φ) in equation (1) is temporally invariant (constant) for a stationary sample besides variations caused by noise. Therefore, small movements of the sample along the optical axis (z) between acquisitions result in a proportional change to the phase of the OCT. Repeated measurements are taken to capture this change in the phase under the assumption that there is only small (sub-point spread function) motion. The change in phase can be measured by repeatedly scanning the beam across the sample at the same location in either 2D or 3D, or by holding the beam stationary, acquiring measurements in time and then moving the beam to another location in 2D or 3D. This concept was first utilized to measure flow in OCT angiography⁶⁸, and its deployment in OCE^{69,70} spurred extensive development. In phase-sensitive OCE, the axial displacement ($u_z(z)$) at the z location of each pixel is²²

$$u_z(z) = \frac{\Delta\varphi(z)\lambda_0}{4\pi n}, \quad (4)$$

where $\Delta\varphi$ is the phase difference calculated between consecutive OCT scans. It should be noted that the phase difference in equation (4) can be calculated between A-scans⁶⁹, B-scans (several A-scans in one frame)⁷⁰ or volumes (several B-scans forming a 3D dataset)⁷¹, depending on the relationship between the deformation being measured and the acquisition time. For example, rapid motion (>200 Hz) in wave-based OCE requires sufficient temporal sampling, so the phase difference is usually calculated between A-scans^{16,64}. However, in compression OCE, the temporal properties of the displacement are not usually utilized, so the phase difference is calculated between B-scans or volumes, depending on whether speed or displacement sensitivity are required for a particular application^{15,64,71}.

A main advantage of phase-sensitive detection is high displacement sensitivity and high spatial resolution, where the resolution matches that of the OCT system used. If the signal-to-noise ratio (SNR) of the detected OCT signal intensity is $\gg 1$, the phase sensitivity ($\sigma_{\Delta\varphi}$) is given by⁷²

$$\sigma_{\Delta\varphi} = \frac{1}{\sqrt{(\text{SNR}_{\text{OCT}})}}. \quad (5)$$

For example, equations (4) and (5) give a displacement sensitivity of 4.7 nm, assuming $\lambda_0 = 800$ nm and a typical SNR_{OCT} of 20 dB. If additionally the noise is zero mean, the phase difference is unbiased; thus, the mean of a large number of displacement measurements from the same pixel provides a reliable estimate of displacement⁷³. In the case that the $\text{SNR}_{\text{OCT}} \ll 20$ dB, the phase sensitivity approaches $\pi/\sqrt{3}$, that is, a uniform distribution of phase differences between $-\pi$ and π ⁷³. However, inaccuracies in dark speckle (dark spots in an OCT speckle pattern caused by random destructive interference of multiple

sub-resolution scatterers) within a focal volume²² (the 3D space defined by the transverse and axial resolutions of the OCT system) should be considered⁷⁴. The maximum measurable displacement is set by the maximum phase difference detectable without ambiguity. Phase describes a 2π angle before showing a singularity and, by applying equation (4) for a refractive index of 1, this corresponds to a maximum displacement of half the mean wavelength λ_0 . The displacement dynamic range can be extended by unwrapping the phase, whereby multiples of 2π are added or subtracted at phase discontinuities to widen the angular range of the phase, typically extending the displacement dynamic range by a factor of 4–5 (ref. 75). The upper limit is ultimately set by speckle decorrelation, which occurs for displacements approaching the axial resolution of the OCT system^{76–78}.

A drawback of phase-sensitive detection is that displacement can only be measured along the optical axis (z). Although this is sufficient in most contemporary OCE techniques that assume a uniaxial load along the z axis, phase-sensitive detection does not allow for the complete characterization of tissue mechanical properties, for which shear components of deformation (transverse to z) should also be measured. In the related field of OCT angiography, this limitation has been overcome, allowing all components of displacement to be measured with phase-sensitive detection. These approaches require either modification to the imaging hardware to allow illumination from multiple angles⁷⁹, or signal processing methods that computationally determine light backscattered from different angles at the expense of spatial resolution⁸⁰. Additionally, artefacts can arise in phase-sensitive detection due to differences in refractive indices in layered samples, which should be corrected for accurate measurements⁸¹.

Correlation-based approaches. In early time-domain OCE studies that pre-dated the widespread use of Fourier domain OCT, displacement was measured by finding the correlation between intensity in local regions of OCT scans^{67,82–85} as the phase was not easily accessible. In time-domain OCT, the envelope of the interference pattern formed the OCT image; thereby, subpixel motion was not accessible via the phase after Fourier transform as in Fourier domain OCT. The most prominent of these methods was speckle tracking, although early OCE studies also used morphological tracking, which is closely related but operates on a larger spatial scale by tracking morphological features in OCT images⁴⁴.

As each speckle realization is determined by the location of scatterers within a resolution element⁷³, if the scatterers in a local region undergo pure translation following mechanical loading (that is, no strain), so too does the associated speckle pattern. This is referred to as the ‘frozen speckle’ model⁸². Thus, the displacement at each location after loading can be found by searching for the location before loading that maximizes a similarity metric, often by cross-correlation. The displacement at each location, $\mathbf{u}(x, y, z)$ is the vector difference between these two locations,

$$\mathbf{u}(x, y, z) = X'(x, y, z) - X(x, y, z), \quad (6)$$

where X' and X are the positions of the local speckle pattern after and before loading, respectively.

An advantage of correlation-based methods over phase-sensitive ones is that deformation can be measured in two or even three dimensions^{86–89}. Speckle tracking remains prominent in situations where 3D displacement is required^{86–88} or phase information is not available, for example, in full-field OCE⁸⁹. Morphological tracking is

less widely used in OCE, but it is still used in applications such as optical palpation^{90–92}.

A disadvantage of tracking methods is the time-consuming iterative methods used to solve for the displacement field, particularly in 2D or 3D. A non-iterative method termed digitally shifted complex cross-correlation can resolve multidimensional displacements⁹³ and has been utilized to obtain the full strain tensor of biological tissue⁹⁴. As an alternative to resolving displacement through speckle tracking, the correlation stability method estimates the relative strain throughout the sample directly from the magnitude of the correlation between OCT intensity scans of the sample before and after loading⁹⁵.

The minimum measurable deformation is determined by the underlying OCT spatial sampling (that is, the voxel size) and has been reported to be 0.5 voxels⁷⁶ in speckle tracking. However, interpolation methods enable subvoxel tracking to within an arbitrary fraction of a voxel^{77,78}, and statistical methods have shown accurate displacement detection down to 0.1 voxels⁷⁷. With complex cross-correlation techniques, the axial displacement sensitivity can even be 3.9 nm (using a window size of 40 μm in an OCT system with 5.5 μm axial resolution and 3.5 μm axial pixel size), corresponding to a sensitivity improvement of $\sim 10\times$ compared with speckle tracking⁹³.

In correlation methods, there exists an inherent trade-off between spatial resolution, accuracy and sensitivity. In general, increasing the window size will increase displacement accuracy and sensitivity at the expense of resolution as the cross-correlation is effectively averaged over a larger area containing more speckle. Typically, the spatial resolution is lower than the OCT resolution by a factor of five to ten⁸³.

OCE techniques

Solid mechanics provide the fundamental relationship between the force imparted to the sample and the resulting deformation^{3,25} necessary for the experimental estimation of sample mechanical properties. The nature of the mechanical load used in OCE determines the appropriate mechanical model to exploit to calculate mechanical parameters. Tissues, cells and biomaterials exhibit a range of mechanical properties, including elasticity, mechanical anisotropy, viscoelasticity and poroelasticity^{1,3,25}, but the sample is often considered as linearly elastic and isotropic to simplify experimental implementation and subsequent computation. As such, the goal in most OCE techniques is to map shear modulus or Young’s modulus²⁵ in a sample, both of which are mechanical parameters measuring the elasticity of the sample^{15,16,64}.

In general, all OCE techniques rely on three main steps: induce displacement, measure displacement and link measured displacement to mechanical properties. In this section, we group OCE techniques into three main categories: compression OCE, transverse wave OCE and axial wave OCE, based on the mechanical model that relates the measured deformation to specific mechanical properties. We also provide an overview of the mechanical loading and models used to estimate elasticity in each case.

Compression OCE. In compression OCE, a low-frequency compressive load is imparted across the surface of a sample^{3,15} as illustrated in Fig. 1a. This is often referred to as quasi-static loading, which implies that the frequency is low enough that inertia is negligible and wave propagation in the sample does not occur^{3,15,25}. Loading is commonly applied with a rigid plate, which is often a glass window to allow imaging and loading from the same side, as shown in Fig. 1a. Often, the interface between the sample and the glass window provides the

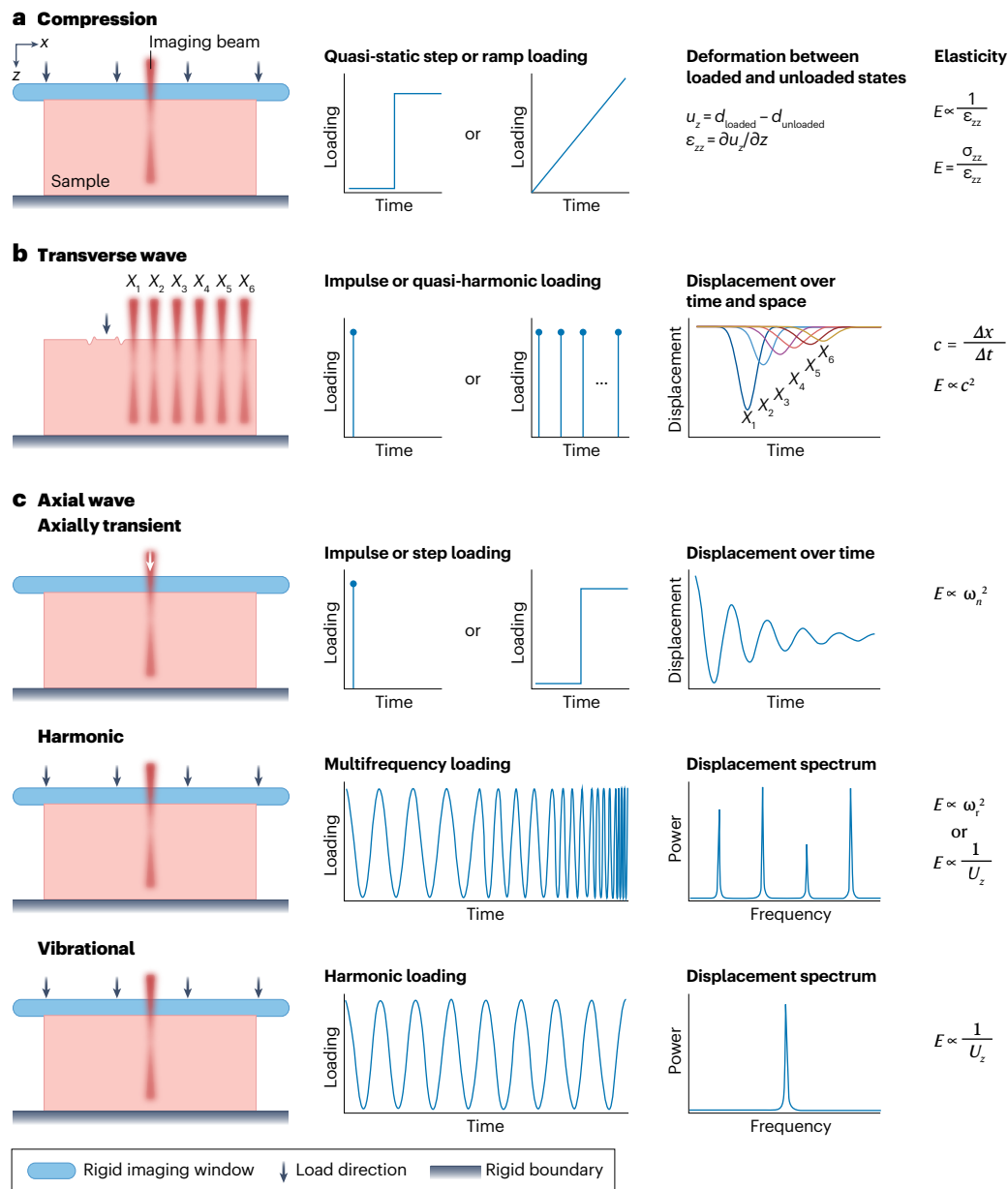


Fig. 1 | An overview of OCE techniques. **a**, In compression optical coherence elastography (OCE), the local displacement (u_z) between two loading states, d_{loaded} and d_{unloaded} , is calculated during quasi-static loading. Elasticity is defined as the ratio of the axial stress, σ_{zz} , to the axial strain, ε_{zz} (equation (7)). **b**, In transverse wave OCE, the displacement (u_z) during wave propagation is measured at different locations (X_n) with synchronized impulse or quasi-harmonic loading. The elasticity is proportional to the square of the wave speed (c), calculated from Δx . **c**, The group of techniques termed axial wave OCE. In axially transient OCE, the local dynamic displacement (u_z) is measured in response to an impulse or a step load. The Young's modulus is estimated by fitting the analytical solution for u_z (usually of a mass-damper system) to the

measured u_z and is proportional to the square of the damped natural frequency, ω_n . In harmonic OCE, the vibration amplitude is spatially mapped under quasi-harmonic loading over a range of frequencies. The elasticity is proportional to the square of the resonant frequency, ω_r , or the reciprocal of the vibration amplitude, U_z . In vibrational OCE, vibration displacement is measured at a single frequency. This is low enough that a similar mechanical model to that used in compression OCE is used to estimate strain or elasticity. In all these techniques, the imaging beam is often scanned and the signal is averaged over the scan, but only a single position is shown for clarity. In the case of transverse wave OCE, multiple positions are shown for ease of explanation. E , Young's modulus.

reference reflection in a common-path configuration, enabling highly stable measurements of displacement by eliminating environmental noise and bulk motion^{15,96}.

The applied load and resulting deformation are described in terms of stress (σ) and strain (ε) tensors, which allow elasticity to be calculated from the constitutive equation linking the two tensors^{3,15,25}. With the

assumption of an isotropic and linear elastic medium under uniaxial loading (only applied along the z direction), the multidimensional solution for elasticity is reduced to one parameter, that is, Young's modulus (E)^{3,15,25}

$$E = \frac{\sigma_{zz}}{\varepsilon_{zz}}, \quad (7)$$

where σ_{zz} and ε_{zz} are the axial components of stress and strain tensors, respectively. These are, by convention, negative in the compressive case and positive in the tensile case. In compression OCE, the goal is to measure σ_{zz} and ε_{zz} to estimate Young's modulus at each pixel in the imaging field of view (FOV). The axial component of the displacement (u_z) is generally measured using phase-sensitive OCT⁶⁹, and ε_{zz} is calculated as the gradient of u_z with depth ($\varepsilon_{zz} = \partial u_z / \partial z$)^{15,97} as shown in Fig. 1a. Using a load cell⁹⁸ or a compliant stress sensor⁹⁰, σ_{zz} is measured at the sample surface. Assuming that the surface stress is uniaxial and distributed uniformly in depth, Young's modulus at each spatial location can be estimated as the ratio of local axial surface stress and local axial sample strain using equation (7) and as shown in Fig. 1a. However, real experimental conditions can deviate notably from this assumption and lead to artefacts, such as inaccurate elasticity, false mechanical contrast, and degradation of spatial resolution and sensitivity^{36,75,76,99–104}, described in detail in the 'Limitations and optimizations' section.

Transverse wave OCE. In transverse wave OCE, a localized and transient stimulation (either impulse or quasi-harmonic) is applied to the sample, and Young's or shear modulus is estimated by imaging the laterally propagating mechanical waves^{3,16} as illustrated in Fig. 1b. In a linear isotropic medium, the elasticity is related to the speed of the transversely propagating shear wave (c_s) by^{3,16,25,105}

$$c_s = \sqrt{\frac{\mu}{\rho}}, \quad (8)$$

where μ is the shear modulus and ρ is the material density. The shear modulus can be expressed in terms of Young's modulus in an incompressible medium as $3\mu = E$, such that $E = 3\rho c_s^2$. Hence, the shear and Young's moduli are proportional to the square of the shear wave speed in transverse wave, allowing quantitative estimation of both parameters from the velocity of the generated waves^{3,16,25}. However, waves that are not pure shear waves, such as Rayleigh or Scholte waves, have a different constant of proportionality relating wave velocity to Young's modulus^{3,16,105}, but the relation remains of the same type ($E \propto c_s^2$) as shown in Fig. 1b. Owing to the relatively shallow imaging depth of OCT, Rayleigh waves are the most common type of wave imaged by OCE in thick samples, and in thin samples such as the cornea, Lamb waves are the most prominent¹⁰⁵.

Loading in transverse wave OCE is typically applied via an external force, such as a focused micro air pulse¹⁰⁶, acoustic radiation force (ARF)¹⁰⁷ or direct indentation⁴⁸. Embedded particles have also been used to generate transversely propagating waves within samples, such as magnetic nanoparticles actuated by an external magnetic field (magnetomotive OCE)¹⁰⁸. Dye-loaded perfluorocarbon nanodroplets have also been used to induce transverse mechanical waves under pulsed laser excitation (nanobomb OCE). The dye absorbs energy from light, and transfers it thermally to the perfluorocarbon, which vapourizes¹⁰⁹. The type of loading should be determined carefully by

considering the order of magnitude of material properties measured (for example, Young's modulus of kPa versus MPa), sample geometry, desired mechanical resolution and other practical considerations, such as patient comfort and tissue accessibility. For example, non-contact loading would be preferred for ocular applications because of patient comfort and ease of alignment. Moreover, the resulting quality of the measurements is influenced by the frequency content of the waves excited by the loading. This is determined by the spatiotemporal properties of the excitation, which can ultimately determine mechanical resolution, contrast and accuracy, as described in the 'Quantitative estimation of mechanical properties' section^{37–39}.

Two emerging approaches for OCE are based on mechanical characterization of a sample by noise correlation, which was pioneered in ultrasound elastography¹¹⁰. One approach, termed diffuse shear wave OCE¹¹¹, uses no external stimulation but rather relies on randomly propagating waves in tissues induced by physiological processes, such as respiration or heartbeat. However, only the local wavenumber can be estimated as the precise frequency of the motion cannot be accurately known with truly passive excitation. Another approach, called reverberant OCE, is based on inducing multiple mechanical waves propagating in multiple random directions and then imaging their resulting interference¹¹². Reverberant OCE was initially demonstrated using a contact-based method, but recent ARF-based non-contact approaches have also been exploited¹¹³. Although promising, the validation studies are ongoing as these techniques are yet to be as well established as other transverse wave OCE techniques.

Axial wave OCE. In axial wave OCE, step, impulse or harmonic loading is applied to the sample, and the time-dependent response along the optical axis is measured, as illustrated in Fig. 1c. Axial wave OCE can be further divided into different techniques based on the type of stimulus exciting mechanical waves in the sample. Axially transient OCE measures the transient response to an impulse or step loading, harmonic OCE measures a frequency-dependent response to continuous multifrequency loading, and vibrational OCE that probes elastic response to sinusoidal (single frequency) loading. Although these constitute distinct OCE techniques, they share similar mechanical loading and models.

In axially transient OCE, free vibration is induced in the sample (loading is applied at $t = 0$) and the sample moves freely without further mechanical loading for $t > 0$ (ref. 114), as shown in Fig. 1c. This is achieved using impulse or step loading^{26,115,116}, and the goal is to fit OCE measurements of the free vibrations to analytical models, often the Kelvin–Voigt model, to extract Young's modulus, which is closely related to the square of the damped frequency by $E \propto \omega_n^2$, and viscosity (η). Most studies have considered an underdamped response, where the damping ratio is between 0 and 1 (refs. 117–120). The damping ratio describes how quickly the oscillations dissipate. For example, multiple oscillations can be seen in the displacement in axially transient OCE in Fig. 1c, which is indicative of an underdamped response. Vibrations have been commonly induced by modulating magnetic nanoparticles impregnated in an external magnetic field gradient (magnetomotive loading)²⁶, ARF^{117,118} or a focused micro air pulse^{119,120}. Overall, the extracted parameters (such as free, or natural, frequencies, which are the dominant frequency of free oscillations) have demonstrated good linear correlation to the square root of elastic moduli in these studies, which is as predicted when modelling tissue as a Kelvin–Voigt material, a common viscoelastic rheological model where the elastic and viscous components are connected in parallel²⁵. However, these frequencies

are also dependent on sample geometry and boundary conditions¹²¹. Another approach is based on a gradient search, where the elasticity and viscosity are altered in the analytical solution of the dynamic displacement until the temporal displacement profile matches that of the OCE-measured displacement profile¹¹⁸. However, this technique requires a priori knowledge of the spatiotemporal properties of the excitation.

In harmonic OCE, continuous sinusoidal loading is applied, as shown in Fig. 1c, and the resulting steady-state displacement is a sinusoid with the same frequency as the excitation but an amplitude that depends on the mechanical properties of the sample, the amplitude of the applied force and the frequency of excitation. Many harmonic OCE studies have reported qualitative measurements of elasticity, typically as the vibration amplitude at a set of frequencies as shown in Fig. 1c. For example, Adie et al. used a piezoelectric actuator driving a compression plate at different excitation frequencies²⁷. Assuming each measured point was independent and loading was provided by equal and uniaxial stress, qualitative stiffness was mapped based on the vibration amplitude (U_z , as shown in Fig. 1c) and phase of the mechanical oscillations (Φ) at selected frequencies. Similarly, Wang and Nuttall used a piezoelectric actuator to probe the cochlea and examined the qualitative contrast in maps of vibration amplitude at 14 kHz and 20 kHz (ref. 24). These studies showed that different excitation frequencies lead to different contrast in regions of varying stiffnesses. Other studies have utilized the sample resonance frequency to generate mechanical contrast. For example, Qi et al. found the resonance frequencies of homogeneous agar phantoms of varying stiffness using ARF excitation¹²². Ahmad et al. demonstrated harmonic OCE using magnetomotive loading across a range of frequencies and mapped the resonance frequency in heterogeneous samples¹²³. Huang et al. extended this technique using faster data acquisition and signal processing¹²⁴. Akka et al. also examined the resonance frequencies and spatial resonance modes of vibrations induced by low-power sound waves in ex vivo bovine corneas¹²⁵.

In vibrational OCE, analogously to harmonic OCE, a continuous sinusoidal mechanical load is applied as illustrated in Fig. 1c. However, vibrational OCE considers the case when the loading frequency is much lower than the sample's natural frequency, and the damping ratio is negligible. This reduces the equation of motion to the purely elastic case (Hooke's law) and enables the use of the same mechanical model as in compression OCE, where displacement is replaced with vibration amplitude, and axial strain is calculated as the axial spatial derivative of the vibration amplitude²⁸. The elasticity $E \propto 1/\epsilon_{zz}$ of compression OCE (Fig. 1a) becomes $E \propto 1/U_z$ in axial wave harmonic OCE in Fig. 1c. In early demonstrations, vibrational OCE was implemented using time domain OCT, which restricted acquisition speed. The technique was later demonstrated using faster spectral domain OCT systems and phase-sensitive detection¹²⁶, enabling volumetric imaging in seconds¹²⁷. As with other forms of OCE, ARF is a common loading method also in vibrational OCE^{128–131}. Most vibrational OCE studies generated only qualitative mechanical contrast. However, if the sample is mechanically coupled to a material of known elasticity, a quantitative estimate of the sample elasticity can be obtained. Guan et al. embedded tendons in pre-characterized agar and mapped the tendon elasticity by quantifying the ratio of vibration amplitude in the sample to the agar¹³². The mechanical resolution and contrast using this method with localized and broad excitation has also been rigorously characterized³⁸ and is discussed in the 'Limitations and optimizations' section.

Passive OCE. An emerging group of passive compression OCE techniques uses mechanical loading by physiological processes^{54,55,133–135}, just as in the diffuse shear wave OCE. These techniques hold the potential to accelerate the clinical translation of OCE by simplifying the experimental hardware, particularly in areas where external loading is challenging, for example, ophthalmology, pulmonology and cardiology. In ophthalmology, proposed passive OCE techniques are generally based on changing intraocular pressure^{133–140}. Passive endoscopic OCE has been used to map tissue deformation in response to fluctuations of natural respiratory pressure^{54,55} and, with further development, may enable in vivo imaging. Similarly, using intravascular OCT to map deformation in response to intraluminal pressure changes⁴⁵ from the heartbeat holds notable potential to translate OCE to applications in cardiology. Despite promising developments, substantial work remains to be done to fulfil the rich clinical potential of passive OCE, where the lack of control of the amplitude and frequency of loading makes it challenging to reliably generate high-quality elastograms. Nevertheless, the first in vivo application of this technique in ophthalmology has been recently demonstrated¹⁴¹.

OCE microrheology. In most OCE techniques, mechanical loading is externally applied to the sample. However, techniques such as magnetomotive OCE^{26,108,123,124,142,143} and photonic force OCE^{59,144} utilize embedded particles for loading. Although the models used in axial wave OCE can be used in this case, there are also mechanical models that are specific to embedded excitation. These models are based on the broader field of microrheology, which studies the flow of embedded particles in fluids or soft solids¹⁴⁵, and are particularly relevant to OCE applications where an active force is applied to embedded particles. So far, OCE microrheology studies have only been conducted using photonic force OCE^{59,144} and ARF¹⁴⁶. However, previous work using camera-based rheology has found the upper limit of loading frequencies for mechanical characterization¹⁴⁷. Understanding this limit is crucial for accurate and robust quantification of mechanical parameters, especially as ultrahigh-frequency OCE approaches have been demonstrated¹⁴⁸.

Results. Here, we describe the steps required to generate elastograms in the two most prominent OCE techniques: compression OCE¹⁵ (Fig. 1a) and transverse wave OCE¹⁶ (Fig. 1b). Readers are directed towards specific publications for details on the data analysis and signal processing of other OCE methods, such as axial transient^{26,115,116}, harmonic^{27,123,125} and vibrational OCE^{128,149}.

Compression OCE

Mapping Young's modulus in compression OCE requires measuring both axial stress and axial strain. Stress was not estimated in early demonstrations, rather it was assumed to be uniform, both laterally and axially, and uniaxial and axial strain was assumed to be inversely proportional to Young's modulus⁶⁷. These assumptions are not strictly true and resulted in inaccurate quantification of Young's modulus and reduced mechanical contrast⁹⁶.

Strain estimation in compression OCE. Axial strain is estimated by computing the gradient of axial displacement between two loading states ($\epsilon_{zz} = du_z/dz$)¹⁵, shown in Fig. 2a,b. Strain is only semi-quantitative unless a stress sensor is used as shown in Fig. 2a–c. Phase-sensitive detection is the most common form of displacement detection, with the phase difference between the unloaded and loaded states being calculated across the entire imaged FOV as shown in Fig. 2d–f. Phase is wrapped to 2π , so phase unwrapping is commonly used to extend the

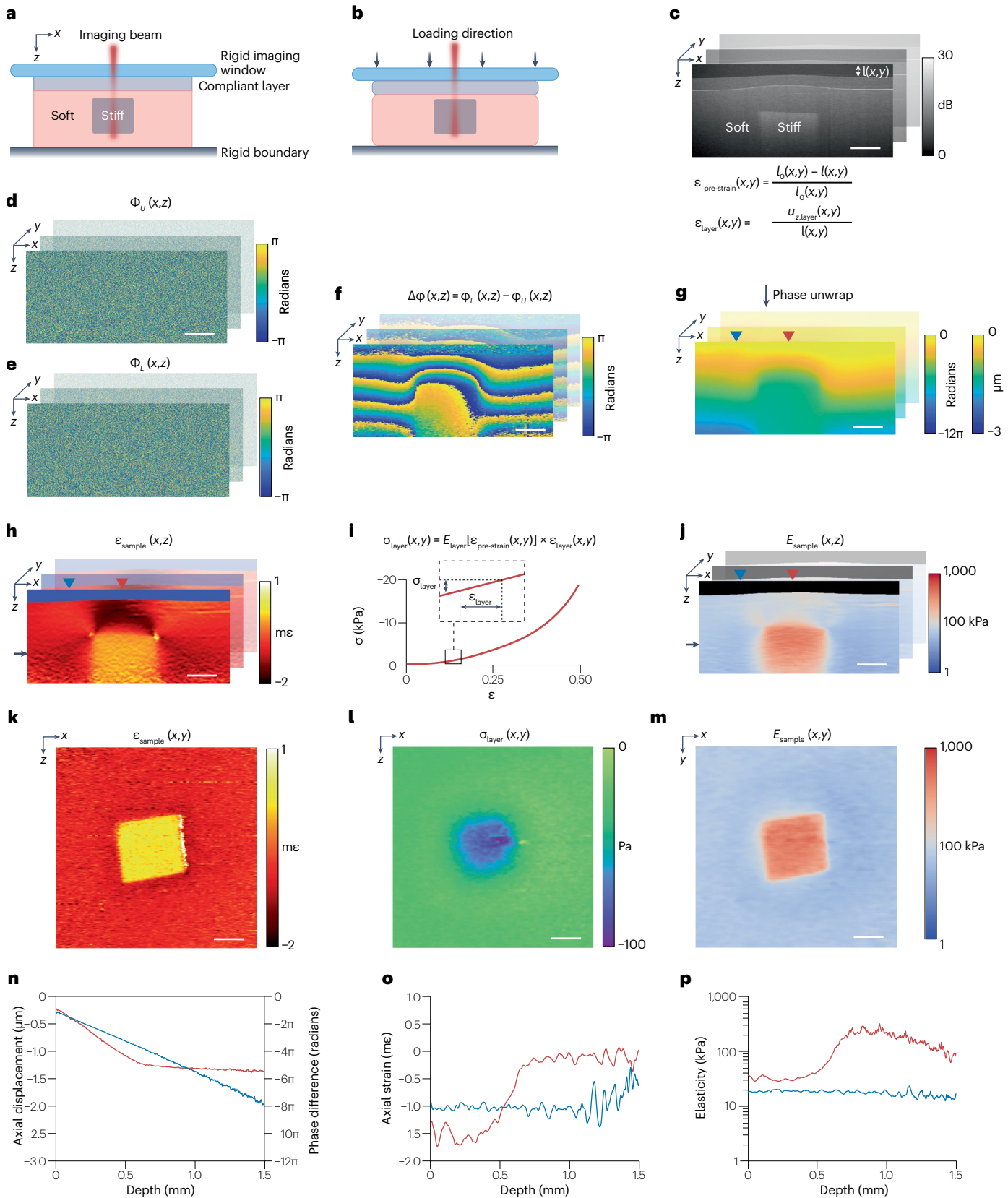


Fig. 2 | Operating principles of phase-sensitive quantitative micro-elastography. **a,b**, Unloaded (part **a**) and loaded (part **b**) configurations for optical coherence tomography (OCT) measurements of a silicone phantom consisting of a stiffer rectangular inclusion in a softer bulk. **c**, OCT B-scans of the compliant-layer and phantom ensemble. Note a bilayered compliant layer is used in this example⁴⁰. **d,e**, Unloaded (part **d**) and loaded (part **e**) OCT phase images. **f,g**, The phase difference ($\Delta\phi$) between the loaded and unloaded frames (part **f**) is unwrapped to extend the dynamic range (part **g**). **h**, Axial strain, ε_{zz} , B-scans computed by using weighted least square linear regression to estimate the gradient of axial displacement with depth⁹⁷. **i**, Stress–strain curve of the compliant layer. **j**, Elasticity B-scans. Elasticity and strain measured in the low

OCT signal-to-noise ratio region of the compliant layer are masked out. **k–m**, En face axial strain (part **k**), en face axial stress measured in the compliant layer (part **l**) and en face elasticity (part **m**). The black arrows in parts **h** and **j** indicate the location of the corresponding en face plane shown in parts **k** and **m**, respectively. **n–p**, Unwrapped phase difference and corresponding axial displacement (part **n**), axial strain (part **o**) and elasticity (part **p**) A-scans where the red and blue profiles correspond to the lateral locations indicated by the blue and red arrow heads in **g**, **h** and **j**, respectively. Each profile is generated from laterally averaging ten A-scans to reduce the noise. Scale bars, 500 μm . Parts **c–h** and **j–p** adapted from ref. 155, CC BY 4.0. Part **i** adapted with permission from ref. 40, OPTICA.

displacement dynamic range and, therefore, strain^{75,96}, as shown in Fig. 2g.

The most straightforward strain estimation approach is the finite difference (FD) method where the ratio of the change in displacement between two depth locations, for example, u_{z1} and u_{z2} , and the physical distance between those points (Δz) is computed by $(u_{z2} - u_{z1})/\Delta z$ ⁶⁷. The main advantage of the FD method is computational efficiency; however, it is very sensitive to noise^{15,97}. Following the precedence in ultrasound elastography, linear regression is applied to the axial displacement over a window of length (Δz) typically 15–100 μm , depending on the OCT axial resolution⁹⁷. This window is then moved along the imaged FOV. Ordinary least squares (OLS) linear regression was first used, where equal weighting was given to each displacement measurement within the window. OLS improves strain SNR compared with the FD method at the expense of lower spatial resolution where the resolution degradation is approximately $\Delta z/\sqrt{2}$ (ref. 97). Weighted least squares (WLS) linear regression improves strain over FD and OLS by weighting axial displacement by the corresponding OCT SNR, which is directly related to the displacement sensitivity, shown in equation (5)^{73,97}. Tissues, such as vessels and ducts, exhibit non-solid regions where strain estimation is invalid and hence they cannot be assumed to be linear isotropic purely elastic media. To partially account for the existence of these areas, an adaptive WLS method was developed^{75,97} (Fig. 2h,k,n,o), further improving elastogram quality¹⁵⁰. Alternatively, the vector method estimates strain from the gradient of the phase difference between consecutive depths in the complex plane¹⁵¹. This method removes the need for phase unwrapping as it relies on FD, where the phase differences are generally much smaller than 2π , so wrapping is not likely to occur, and strain SNR can be improved by summing the complex phase difference gradient axially and laterally, effectively weighting phase difference measurements by the OCT intensity, similar to WLS. Although WLS typically exhibits the highest strain SNR due to the weighted linear regression over a relatively larger kernel as compared with the vector method, the lower complexity of the vector method significantly reduces computation time. For example, the computation time for WLS and the vector method was approximately 146 s and 22 s, respectively, when processing the same 3D compression OCE data⁷⁵. Recent demonstrations have shown real-time (~20 Hz) OCE with the vector method¹⁵². In addition to estimating axial strain, mapping the full strain tensor has been demonstrated using several methods including cross-correlation⁹⁴, digital volume correlation^{87–89} and an image-based inverse reconstruction method¹⁵³.

Elasticity imaging in compression OCE. Several methods have been developed to estimate both axial stress and axial strain to quantify Young's modulus by equation (7). Assuming uniaxial stress, these

methods use either the deformation of a pre-characterized material to map 2D axial stress at the sample surface⁹⁰ as shown in Fig. 2a–c, or measurements of point or bulk stress from a force sensor^{98,154}. In an early demonstration of axial stress mapping, known as optical palpation, a transparent, compliant layer with a pre-characterized stress–strain relationship, such as that shown in Fig. 2i, was placed between the sample and the imaging window⁹⁰. As the stress–strain relationship is known, the measured strain of this layer at each lateral point is used to obtain the corresponding stress as shown in the inset in Fig. 2i,l. Optical palpation is a qualitative elastography technique^{91,92}, which has been combined with strain imaging methods – described in the previous section – in a technique referred to as quantitative micro-elastography (QME), where Young's modulus is mapped in 3D, as illustrated in the orthogonal planes¹⁵⁵ shown in Fig. 2j,m. Similarly to optical palpation, in QME a transparent compliant layer is placed between the sample and imaging window (Fig. 2a–c). A bulk strain is then applied to the layer–sample ensemble to ensure uniform contact, and an additional periodic stepwise loading as shown in Fig. 1a is applied to the whole system to map elasticity of the sample. The stepwise loading enables rapid volumetric imaging, while ensuring that the tissue is at the same bulk strain throughout the data acquisition. Owing to the high displacement sensitivity of phase-sensitive detection, QME also has a very high elasticity sensitivity of ~1 kPa (ref. 102). Alternatively, a ramp load can be applied in a QME measurement¹⁰¹. The ramp loading approach can readily map nonlinear elasticity and provides greater capacity to account for non-uniform loading in samples with uneven surfaces. However, these methods have been limited to 2D imaging because they require the acquisition of a large number of B-scans (~200–400) at each location^{34,156}. Quantitative OCE techniques that obtain a point estimate of axial stress include those based on force sensors^{154,157–159} and deformable silicone spokes with pre-characterized force-displacement curve attached to a glass window⁴². Although these methods require the assumption of a uniform stress laterally across the sample surface, they provide a straightforward means to estimate stress practically, such as in handheld probes^{42,157,160}.

The iterative solution to the inverse elasticity problem in compression OCE. The assumptions of isotropy and uniaxial deformation made in compression OCE that lead to equation (7) can result in erroneous estimations of elasticity as complex samples such as biological tissues often deviate from these assumptions. A more general mechanical model and an iterative solution to the inverse elasticity problem can dramatically reduce these errors^{153,161–163}. In these instances, the elasticity distribution is numerically modelled, the displacement is then computed depending on the sample geometry and the boundary conditions, and compared with the OCE-measured displacement. Then, the elasticity in the model is updated to minimize this difference within an acceptable tolerance.

When the target tolerance is met, the computed elasticity is assumed to be the best estimate of the true elasticity of the sample. Preliminary results demonstrate improved accuracy and spatial resolution over conventional compression OCE^{153,163}. Despite the complexity of tissue, iterative methods that use finite element modelling (FEM) and regularization can provide meaningful results when combined with models that account for different tissue behaviour, for example, nonlinearity, heterogeneity and anisotropy. However, the models need to be carefully chosen to accurately represent tissue behaviour to mitigate the risk of an ill-posed solution. It could happen that multiple displacement fields produce the same elasticity, so that the choice of the solution would be ambiguous. Additionally, computational requirements restrict real-time imaging applications (for example, intraoperative use) but deep learning can probably overcome this restriction^{61,164,165}.

More complete estimation of mechanical properties in compression OCE. As compression OCE and its applications mature, greater emphasis is being placed on estimating additional mechanical properties beyond linear elasticity. For example, correlation-based approaches have been utilized to estimate the full strain tensor, providing information on mechanical anisotropy^{87–89,93,94,153,166}. In addition, compression OCE has mapped mechanical properties such as nonlinear elasticity¹⁵⁷ and viscoelasticity¹⁶⁷. Although these techniques typically require longer acquisition times, larger datasets and longer processing times, we anticipate an acceleration in their development and new applications.

Transverse wave OCE. In transverse wave OCE, the shear modulus is estimated by measuring the speed of the propagating mechanical wave¹⁶, typically using phase-sensitive detection. Crucially, a priori knowledge of the applied stress is not required for mechanical quantification, making transverse wave OCE an attractive solution in many applications^{3,16}. Early methods assumed the mechanical waves were pure shear waves, but this is now understood to be largely untrue, and the development and application of more accurate and complex mechanical models has been a major focus of recent transverse wave OCE development^{168–171}. We describe commonly used data processing steps used to convert measured displacements into wave propagation characteristics and algorithms for de-noising the OCE data.

Filtering and autocorrelators in transverse wave OCE. If the mechanical loading is quasi-harmonic (that is, multiple cycles at a single frequency), traditional filtering of the temporal displacement, such as finite impulse response filters, can tremendously improve the SNR of the detected wave motion. However, special care should be taken to utilize zero-phase techniques because the phase of the detected motion (not to be confused with the phase of the OCT signal) is critical for quantifying mechanical parameters, such as wave speed^{16,64}. A common zero-phase filtering method relies on temporally filtering the signal forwards and backwards to cancel out phase shifts induced by filtering itself. Beyond these methods, autocorrelators can retrieve motion and improve its SNR, particularly in regions of low OCT SNR¹⁷², and the Kasai¹⁷³ and Loupas¹⁷⁴ methods are the most prominent. These methods are closely related, where the Loupas method incorporates space as an additional domain to improve the displacement SNR by effectively locally averaging displacements that are weighted by the OCT SNR.

Calculating elastic wave group velocity. Wave speed is calculated from either the group velocity or phase velocity using a variety of

methods^{16,64}. In the general case, the group velocity includes all the frequencies contained in the local wave packet, and phase velocity is related to the local wave packet's single-frequency components. For quasi-harmonic excitation, they are the same. When using the group velocity for mechanical characterization, special care must be taken to ensure that assumptions such as a non-dispersive and sufficiently thick sample – relative to the wavelength of the elastic wave – are valid¹⁷⁵. The most widely used methods to quantify group velocity rely on peak tracking and cross-correlation¹⁶. In the former, a singular peak is tracked along the wave propagation path to obtain the propagation lags at known distances, which is utilized in both impulse and quasi-harmonic loading. In the latter, stationary temporal displacement profiles based on impulse stimulation are cross-correlated with a template profile (Fig. 3a,b) to obtain the wave propagation lags at the known distances of each profile position¹⁷⁶. With quasi-harmonic loading, this method can be unreliable as cross-correlation of a quasi-harmonic sinusoid with another can result in ambiguity when determining the wave propagation lag. Nevertheless, the speed is estimated by fitting the propagation lags to the corresponding propagation distances with both peak tracking and cross-correlation methods, typically using least squares regression, as illustrated in Fig. 3c.

Calculating elastic wave phase velocity. The phase velocity refers to the wave speed at a single frequency. It can be calculated either through single-frequency quasi-harmonic stimulation or by filtering a broadband displacement resulting from impulse stimulation. In the former case, phase velocity can be retrieved by the analogous procedure followed for group velocity, as the travelling mechanical wave packet is composed of a single frequency. For a broadband displacement signal, traditional temporal filtering can be used, such as a narrowband finite impulse response filter centred at the frequency of interest. For example, the wave speed of a filtered broadband wave induced by a focused air pulse was shown to be equal to the wave speed for a narrowband quasi-harmonic wave induced by air-coupled ultrasound (ACUS) in phantoms, ex vivo rabbit corneas and in vivo human corneas¹⁷⁷. To quantify the dispersion of a broadband wave and retrieve its phase velocity, far more computationally efficient methods are available. Most prominently, a 2D fast Fourier transform can be performed on a space-time map (x, t) of the measured displacement (Fig. 3d) to obtain a corresponding wavenumber-frequency map (k, ω) of the wave propagation^{16,64} (Fig. 3e). The wavenumber of the peak at each frequency in the $k - \omega$ map is used to calculate the corresponding wave speed at that frequency as $c(\omega) = \omega/k_{\text{peak}}(\omega)$ to obtain the final dispersion curve shown in Fig. 3f.

Reverberant and diffuse shear wave OCE. Signal processing in reverberant and diffuse shear wave OCE is vastly different from other methods as these methods rely on noise correlation approaches, rather than directly quantifying wave propagation. For these OCE techniques, the spatiotemporal autocorrelation of the axial deformation is calculated within a localized kernel and is then either fitted to an analytical solution for reverberant OCE¹¹² or it is related to the local strain for diffuse shear wave OCE¹¹¹. In both cases, the local wavenumber (k) is obtained and used to retrieve the local shear wave speed ($c_s = \omega_s/k$), if the frequency of the stimulation (ω_s) is known.

Applications

OCE has been applied to characterize a wide range of tissues and is also emerging as a technique to characterize cells and biomaterials. Here,

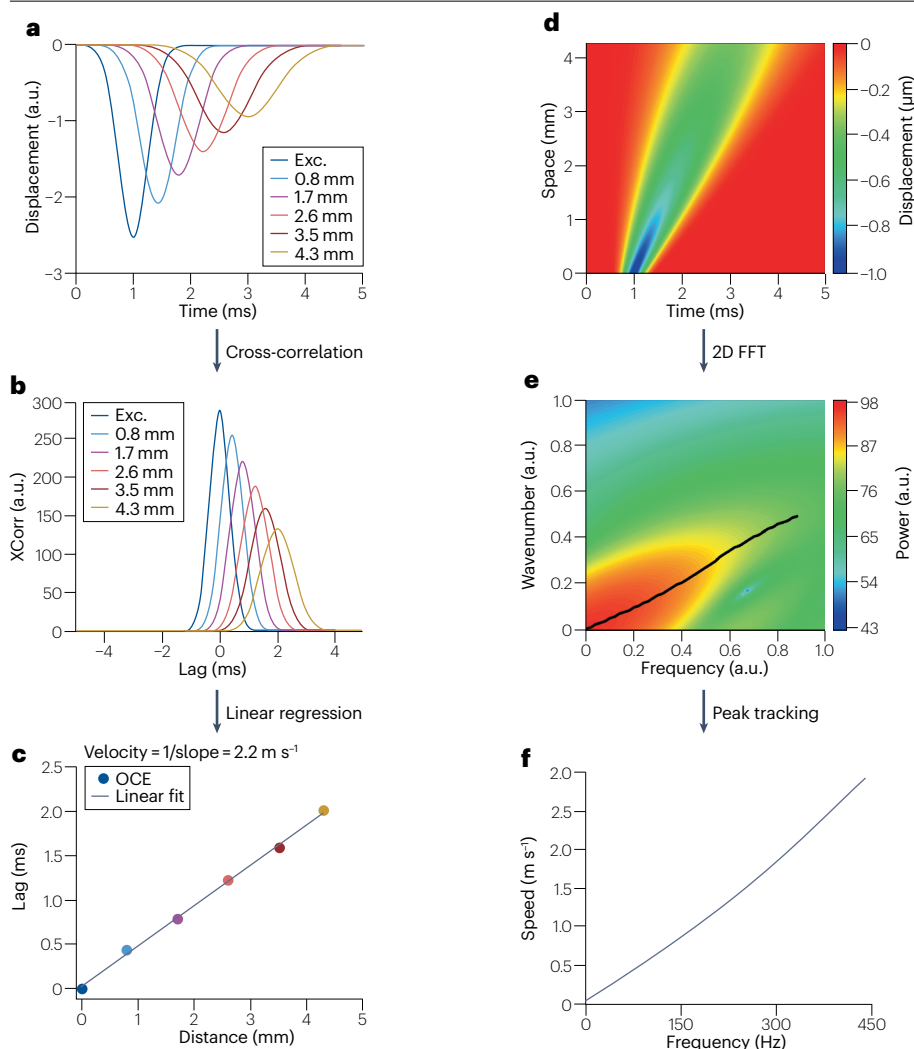


Fig. 3 | Velocity calculation methods in transverse wave optical coherence elastography. **a–c**, Group velocity calculations: the sample stationary displacement profiles at the indicated distances from the excitation (Exc.) (part **a**) and their cross-correlation with the excitation profile (part **b**), and propagation lags between the tracked peaks of the cross-correlation (XCorr) and their fit to obtain the group velocity (part **c**). **d–f**, Phase velocity calculations: a space–time map (part **d**) of the displacement induced by the wave propagation and a wavenumber–frequency map obtained by two-dimensional fast Fourier transform (2D FFT) (part **e**). The solid black line shows the peak wavenumbers at each frequency used to calculate the dispersion of the mechanical wave (part **f**). a.u., arbitrary units; OCE, optical coherence elastography.

we highlight areas that have been the focus of OCE and where there has been notable progress resulting, in some cases, in successful clinical translation to studies on patients.

Ophthalmology

The onset and progression of many eye diseases are linked to pathological changes in the mechanical properties of ocular tissues. For example, softening of the cornea can lead to keratoconus, resulting in a conical shape and subsequent loss of visual acuity¹⁷⁸. A clinically approved mechanotherapy, crosslinking (CXL), stiffens the cornea and halts the progression of keratoconus¹⁷⁹. Cataract and presbyopia have been linked to stiffening of the crystalline lens¹⁸⁰, so prospective treatments are focused on softening it¹⁸¹. Clearly, mechanical characterization of ocular tissues would enable early disease detection, treatment planning and therapy evaluation. To this end, OCE has been developed and implemented in several ophthalmic applications²⁹. Recent approaches have leveraged the high displacement stability of OCE in the cornea to measure minute displacements caused by pressure differences, either externally^{135,182} or internally, for example, intraocular pressure^{133,134,138–140,183}. For example, Fig. 4a–c shows mid-stromal

strain in ex vivo rat corneas induced by ambient pressure modulation for central (Fig. 4a), peripheral (Fig. 4b) and hourglass-patterned (Fig. 4c) CXL¹⁸². However, transverse wave OCE remains the most common approach for measuring ocular biomechanical properties. Although non-contact stimulation in the eye is preferred, contact-based methods have shown no induced damage and the ability to map wave speed and shear modulus in the sclera, limbus and cornea in human individuals^{184,185} (Fig. 4d). Nevertheless, a focused air pulse¹⁰⁶ has been a common approach in both transverse wave OCE and axially transient OCE, including in vivo in preclinical animal models^{186,187}, healthy human individuals^{119,120,188–190} and patients with keratoconus^{30–32} owing to easy access to the anterior eye. Another common non-contact stimulation approach is ACUS. Figure 4e shows the propagation of a Lamb wave induced by a line-focused ACUS transducer (that is, acoustic micro-tapping¹⁹¹) in an ex vivo porcine cornea at various times after excitation at a controlled intraocular pressure. Combined with a transverse isotropic mechanical wave model, acoustic micro-tapping has been used to characterize the elastic anisotropy of the cornea¹⁶⁸ and the layer-wise changes caused by CXL¹⁹². Confocal ACUS excitation has also been utilized to assess the changes in corneal stiffness due to CXL,

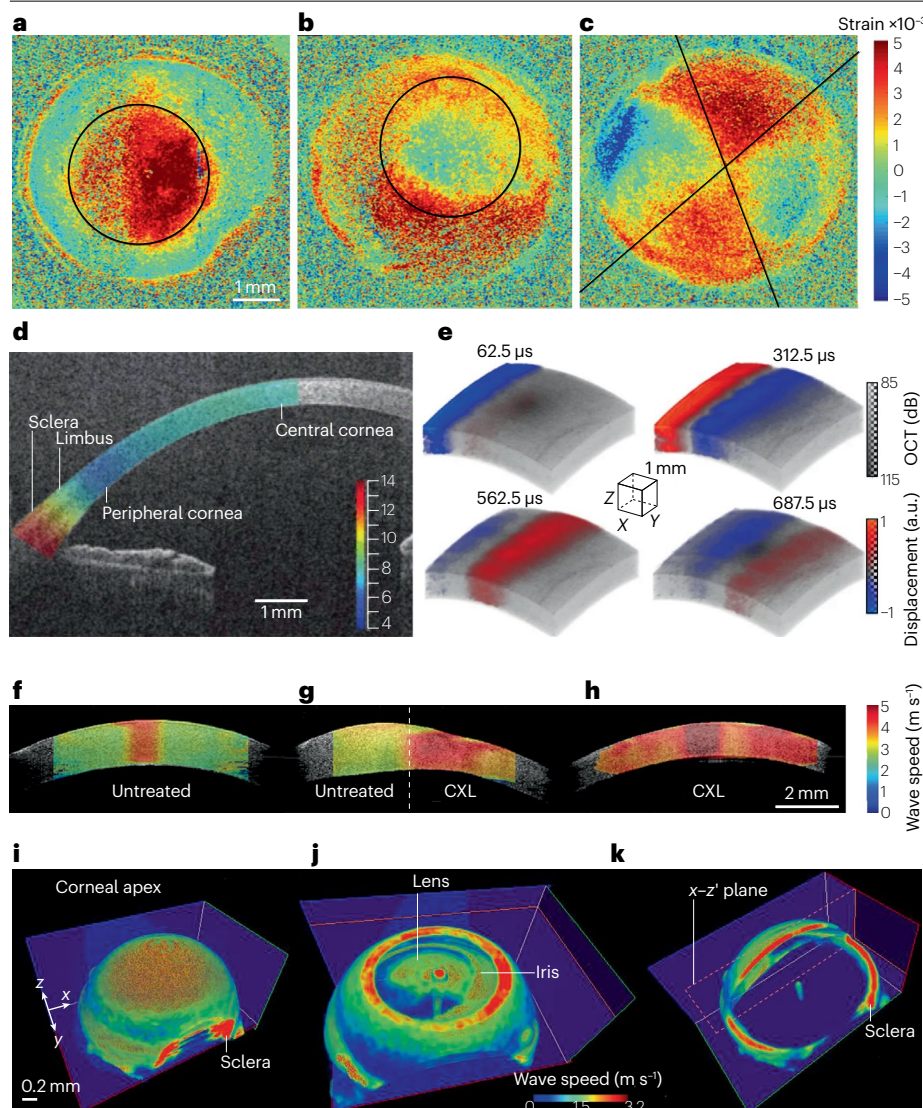


Fig. 4 | Examples of optical coherence elastography applications in ophthalmology. **a–c**, Mid-stromal axial strain during ambient pressure modular of ex vivo rat eyes after central (part **a**), peripheral (part **b**) and hourglass-shaped (part **c**) patterned crosslinking (CXL)¹⁸². **d**, Wave speed at 16 kHz across the sclera, limbus and cornea in a human individual¹⁸⁵. **e**, Propagation of a Lamb wave induced by acoustic micro-tapping in an ex vivo porcine cornea at the noted times after excitation¹⁹¹. **f–h**, Lamb wave speed elastogram of ex vivo untreated (part **f**), half-CXL-treated (part **g**) and traditional CXL-treated (part **h**) porcine corneas¹⁹³. **i–k**, Mapping of the stiffness of a whole ex vivo murine eye globe with water-coupled acoustic radiation force-based reverberant optical coherence elastography showing various cuts. A slight cut in the *x–z* plane shows the sclera (part **i**), an en face cut in the *x–y* plane shows the lens and iris areas (part **j**) and partial planar cuts show depth and en face mapping of the sclera, simultaneously (part **k**)¹⁹⁵. a.u., arbitrary units; OCT, optical coherence tomography. Parts **a–c** adapted with permission from ref. 182, Royal Society. Part **d** adapted with permission from ref. 185, IEEE. Part **e** adapted from ref. 191, CC BY 4.0. Parts **f–h** adapted with permission from ref. 193, OPTICA. Parts **i–k** adapted from ref. 195, CC BY 4.0.

and Fig. 4c shows the speed of confocal ACUS-induced Lamb waves in ex vivo porcine corneas, which highlights the ability to detect the transition between the untreated and CXL-treated regions in a half-CXL cornea¹⁹³. Reverberant approaches, albeit with contact, have also been utilized to map corneal mechanical properties^{112,194} and, recently, an ARF-based reverberant method was used to map the stiffness of a whole mouse eye globe ex vivo¹⁹⁵ (Fig. 4i–k). ARF is preferred for mechanical characterization of internal ocular tissues, such as the iris¹⁹⁶, lens¹¹⁸ and retina¹³⁰ owing to its ability to induce displacements inside tissue remotely. The mechanical properties of the sclera have also been characterized by transverse wave OCE^{197–199}.

Although most OCE applications in ocular tissues have been wave based²⁹, compression OCE also has the potential to assess a range of conditions in the eye, most notably in the cornea. Early demonstrations of compression OCE in the cornea of ex vivo animal models validated that CXL increases cornea stiffness^{89,200,201}. Compression OCE also showed that thermomechanical laser-assisted reshaping can selectively soften regions of the cornea²⁰². To address potential discomfort from

contact-based loading, several OCE techniques have explored passive loading. For example, changes in intraocular pressure have been used to impart deformation in the cornea in ex vivo studies^{133,134,136–138,140,203} and in vivo preclinical research based on the heartbeat-induced ocular pulse^{137,181}. A non-contact compression OCE technique, which uses changes in ambient pressure via sealed goggles, has been demonstrated in vivo in humans¹³⁵.

Oncology

It is well established that cancer changes the micro-scale mechanical properties of tissue, providing a biomarker for the identification of tumours²⁰⁴. This is already widely exploited clinically by using ultrasound elastography⁴ and magnetic resonance elastography⁵. The clinical potential of OCE has been demonstrated in several areas of cancer imaging, most notably using compression OCE^{33,155,205}. Extensive development has been focused on intraoperative tumour margin assessment in breast-conserving surgery^{33,35,206} to assist surgeons in removing cancer more effectively during surgery, which would reduce

high re-excision rates (by approximately 20–30%²⁰⁷). A diagnostic accuracy study on 90 patients using QME reported sensitivity and specificity of 93% and 96%, respectively, for detecting cancer within a 1 mm margin³³. In vivo QME using a handheld probe has also been demonstrated to detect residual cancer in the surgical cavity after excision of the primary tumour³⁵. Figure 5A–F shows representative QME images of excised cancerous breast tissue¹⁵⁵, where the tumour is much stiffer than the surrounding tissue. Compression OCE can also distinguish between different life stages of tumorous cells based on the statistical distribution of elasticity, referred to as ‘elasto-spectroscopy’³⁴, as shown in Fig. 5G–I. In addition to handheld imaging probes, both needle probes^{208,209} and finger-mounted probes²¹⁰ have been demonstrated, with the main goals of increasing the imaging depth achievable in breast tissue and improving ease-of-use during surgery, respectively. Clinical studies have also demonstrated the potential of compression OCE in colorectal cancer detection²¹¹. Ex vivo studies have demonstrated the potential of compression OCE to measure stiffness changes caused by cancer in the pancreas²¹², liver²¹³, brain²¹⁴ and ovary²¹⁵ and for assessing drug response²¹⁶. Vibrational OCE has been proposed as a means of biopsy guidance¹⁴⁹ and margin assessment²¹⁷ in prostate cancer, shown in Fig. 5J–L, with a sensitivity and specificity of 89.6% and 99.8%, respectively. Magnetomotive OCE has assessed elasticity changes in soft tissue sarcoma¹⁴² and melanoma¹⁴³. For example, Fig. 5M–O shows stiffening of a soft tissue sarcoma after thermal therapy, where magnetic nanoparticles were used for inducing mechanical waves and for thermal therapy in a theranostic application¹⁴².

Cardiology

Blood vessel elasticity is a key regulator of cardiovascular function^{1,218}. In atherosclerosis, plaque deposition and pathological changes in arterial wall elasticity are linked with hypertension and stroke, and gradual arterial stiffening with age is associated with an increased risk of cardiovascular disease²¹⁹. To this end, OCE has been steadily developed with the goal of mapping the micro-scale mechanical properties of blood vessels to better understand cardiovascular biomechanics and to identify atherosclerotic plaques in vivo. Several applications of OCE have shown biomechanical mapping of ex vivo vessels^{44–46,156,220,221}. OCE based on a high-resolution variant of OCT, optical coherence microscopy (OCM), resolved the elasticity of individual lamellae and variations in vascular smooth muscle in murine arteries²²⁰, and digital volume correlation has been used to map radial, axial and circumferential strain in porcine arteries, highlighting the mechanical anisotropy of vessels⁴⁶. OCE has also been used to quantify the elasticity of ex vivo human pericardium tissue towards improving surgical outcomes of aortic valve leaflet replacement¹⁵⁶, to assess the radial elasticity of ex vivo human great saphenous vein tissue²²¹, to study the biomechanics of myocardial infarction²²² and to characterize mitral valve elasticity²²³. Several intravascular OCE probes have been developed for in vivo translation^{43–45}. Recently, intravascular OCE has shown a stark contrast in radial strain between calcified atherosclerotic plaques and surrounding healthy tissue in ex vivo human coronary arteries using a catheter-based probe⁴⁵.

Dermatology

The skin undergoes repeated and large mechanical loading and unloading, and numerous pathologies affect its mechanical properties, such as systemic sclerosis²²⁴. Dermatology was proposed as one of the earliest applications of OCE in human individuals, with early studies using mechanical actuators to create mechanical waves^{48,49} and imaging audio frequency vibrations²⁸. Since then, notable progress has been made

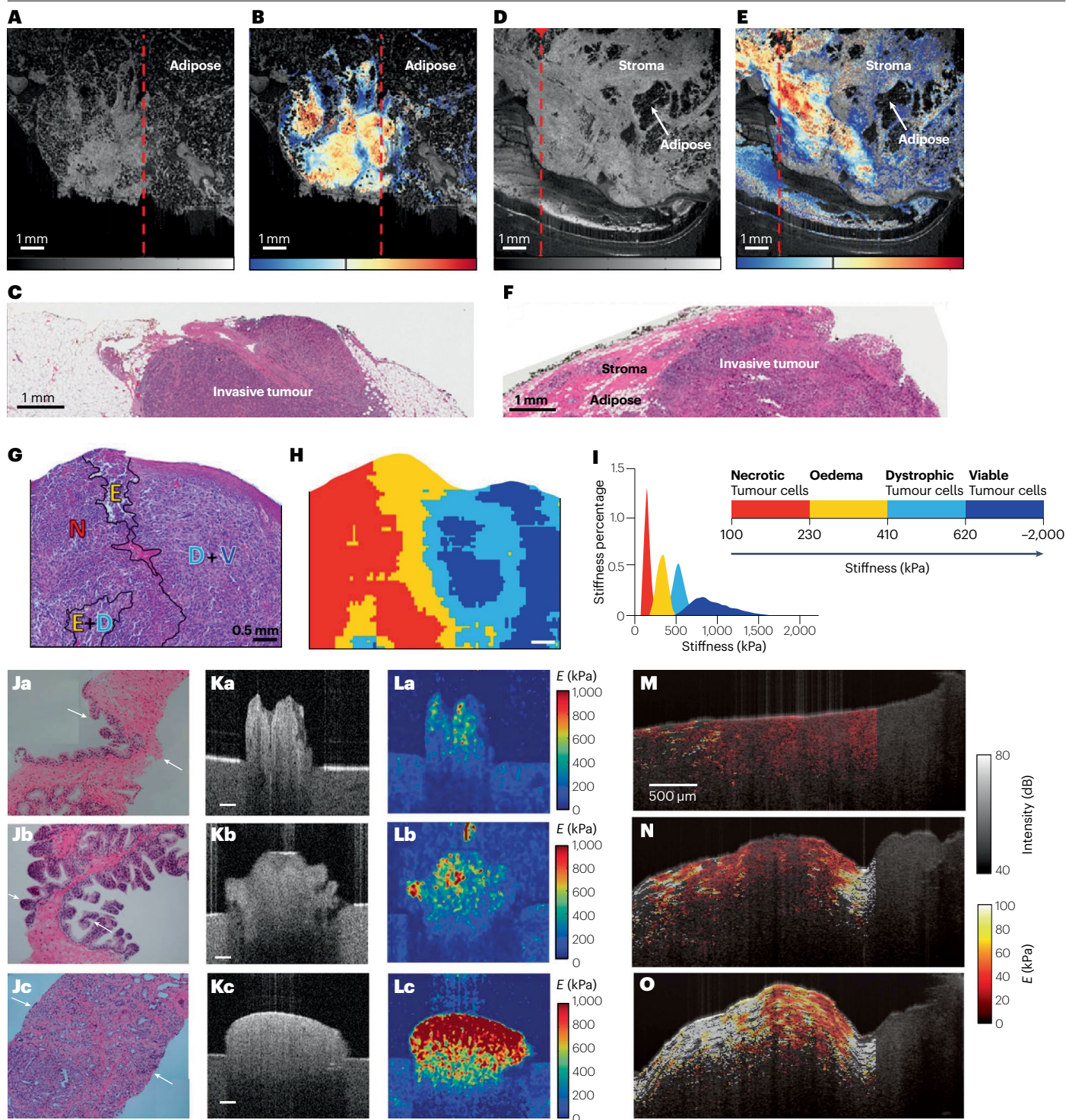
in the applications of OCE in dermatology. For example, transverse wave air-pulse OCE can measure dermal fibrosis in systemic sclerosis in patients as well as a trained clinician’s manual palpation⁵³. This work is built upon earlier transverse wave air-pulse OCE studies showing how fibrosis stiffens mouse skin ex vivo and in vivo⁵⁰. More recently, high-frequency contact-based excitation and a layered model have been used to quantify the layer-wise mechanical properties of normal skin in human individuals⁵². Alternatively to the air-pulse stimulation, acoustic micro-tapping has also been used in combination with a transversely isotropic model to characterize the mechanical anisotropy of the skin in normal human individuals and to localize stiff scar tissue⁵¹.

Mechanobiology

Physical forces and mechanical properties are increasingly recognized as key regulators of cell function and disease²²⁵. Although studies in mechanobiology most commonly characterize cells in 2D biomaterials, growing research suggests the importance of studying cells in 3D biomaterials that more accurately replicate physiological conditions^{226,227}. A main advantage of OCE over established 2D techniques, such as AFM¹¹, is the ability to provide 3D characterization of cells in 3D biomaterials. In an early demonstration of compression OCE in mechanobiology, the axial strain of a 3D collagen matrix embedded with cells was characterized⁸⁵. Following the development of quantitative techniques, OCE has been applied to characterize biomaterial elasticity^{159,228–232} and cellular elasticity in both single cells⁵⁷ (Fig. 6a,b) and cellular spheroids⁶⁰. Compression OCE has also been used for subcellular imaging in a OCM-based realization, termed mechano-microscopy^{58,61} (Fig. 6c–e). Recently, multimodal mechano-microscopy with integrated confocal fluorescence microscopy showed that the mechanical phenotype of metastasizing tumour spheroids is mediated by the elasticity of the microenvironment²³³ (Fig. 6f–j). Photonic force OCE has also been utilized to characterize the elasticity of cells and their surroundings in 3D^{59,144}. For example, Fig. 6k,l shows localized stiffening of the extracellular matrix around cells, which is not present when the cells were treated with an actin polymerization inhibitor in Fig. 6m.

Emerging applications

Beyond the more established OCE applications, several new areas are emerging. For example, Fig. 7a,b shows that reverberant OCE has promise for mapping the mechanical properties of small mammal embryos with resolution below 100 μm and FOV of a whole post-implantation mouse embryo²³⁴. Transverse wave OCE has also been used to characterize the mechanical properties of ex vivo murine kidneys²³⁵ (Fig. 7c), colon specimens²³⁶, liver^{170,237} and genitourinary tissues^{149,238}. Compression OCE has been applied to study muscular dystrophy, characterizing ex vivo murine skeletal muscle tissue in models for Duchenne muscular dystrophy²³⁹ and dysferlinopathy²⁴⁰, as shown in Fig. 7d–i. Compression OCE has also been used to characterize vaginal wall tissue showing the correlation between tissue elasticity, collagen thickness and collagen orientation²⁴¹. Emerging OCE techniques based on tension characterization have been developed because the function of many load-bearing tissues is regulated by local tensile mechanical properties²⁴². Key findings from these studies include that local strain can be an order of magnitude greater than that of the applied bulk strain and that the largest strain component can often be in a direction other than that of the applied load^{47,150}. OCE has also been applied to measure the stiffness of the trachea, where the cross-sectional compliance (that is, deformation) in ex vivo porcine tracheas was measured with a catheter-based probe²⁴³, as illustrated in Fig. 7j. The results showed that



steam injury decreased the cross-sectional compliance, corresponding to an increase in stiffness.

Reproducibility and data deposition

Given the focus on technical development of the extensive range of demonstrated OCE techniques, little effort has been put into creating

widely available or accepted standards for OCE. However, OCE techniques are generally first validated on phantoms, making phantoms critical for the validation and characterization of OCE techniques. Therefore, the specifications and fabrication procedures of these phantoms are normally well documented. Two commonly used materials for tissue phantoms are gelatin^{38,42,52,56,67,83,106,107,112,113,115,117,121,170,172,187,210,244,245}

Fig. 5 | Applications of OCE in oncology. **A–F**, Compression optical coherence elastography (OCE) of human breast cancer: optical coherence tomography (OCT) (part **A**), quantitative micro-elastography (part **B**) and histology (part **C**) of highly cellular invasive ductal carcinoma at the surgical margin (indicated by the red dashed lines in parts **a** and **b**), and OCT (part **D**), quantitative micro-elastography (part **E**) and histology (part **F**) of invasive ductal carcinoma at the surgical margin (indicated by the red dashed lines in parts **d** and **e**) surrounded by adipose tissue and stroma. The colour bar ranges show OCT of 0–40 dB and elasticity of 3.63–363 kPa (ref. 33). **G–I**, Elasto-spectroscopy of human breast cancer: histology (part **G**) and segmentation (part **H**) of the elastogram into different evolution stages of a treated cancerous tissue – necrotic (N), oedema (E), dystrophic (D) and viable (V) – using the ranges of elasticity – measured as the

tissue stiffness, in kPa – shown in part **I**³⁴. **J–L**, OCE of human prostate cancer: histology (**Ja**), OCT intensity (**Ka**) and corresponding elasticity (**La**) of benign prostate tissue; prostatic intra-epithelial neoplasia (**Jb**, **Kb** and **Lb**, respectively); and malignant prostate cancer (**Jc**, **Kc** and **Lc**, respectively) with a Gleason score of 4 + 3 (ref. 217). Arrows in part **J** indicate the boundaries of the tissue of interest. Scale bar, 200 μ m. **M–O**, OCE of canine soft tissue sarcoma: OCT intensity images and overlaid elasticity maps before treatment (part **M**), after the first treatment (part **N**) and after the second interstitial magnetic thermotherapy treatment (part **O**) with magnetic nanoparticles¹⁴². **E**, Young's modulus. Parts **A–F** reprinted with permission from ref. 33, AACR. Parts **G–I** adapted from ref. 34, CC BY 4.0. Parts **Ja–Lc** adapted with permission from ref. 217, Elsevier. Parts **M–O** adapted with permission from ref. 142, OPTICA.

and agar^{38,49,81,108,109,122,129,158,176,193,237,246,247} owing to their mechanical tunability, wide availability and overall broad use in the elastography field³. Gelatin is often chosen for its low viscosity¹⁷⁰ and linear response to mechanical loading, thereby making elasticity quantifications simpler and more easily reconcilable with mechanical testing. When accurate evaluation of viscoelastic mechanical models is required, agar is the material of choice as it has notably greater viscosity than gelatin. Silicone phantoms are also commonly used^{126–28,41,44,46,48,52,71,74–76,89,90,94–97,99,102,103,116,123,124,126,136,142,153–155,157,160,162,164,166,167,206,208,212,220,233,243,248} owing to their high linearity (except at high strains^{90,155,157}) and permanence, which is in contrast to gelatin and agar phantoms that usually need to be recreated for every experiment. Although silicone does have notable viscosity¹⁶⁷, silicone phantoms do not dehydrate, contrarily to gelatin, agar, agarose and other gel-based phantoms where this happens commonly, resulting in measurement errors. Another important advantage of silicone is its suitability for moulding, making it straightforward to fabricate heterogeneous phantoms comprising regions of different elasticity²⁴⁹ and also to mimic specific tissue features, such as surface roughness²⁴⁸. Other materials such as polyvinyl alcohol^{36,37,39,40,43,45,69,70,84,163,175,214} have also been used but are often more complex to generate. Regardless of the type of phantom, optical scattering agents, most commonly titania particles (TiO₂), are added for optical contrast.

We anticipate and encourage the development of well-calibrated and stable phantoms that are distributed among research groups working on each of the main OCE techniques. In addition to providing an important means to assess the reproducibility of OCE data, this approach would enable the assessment of the imaging performance of the various techniques and would aid in the selection of a particular technique for a given application.

Most OCE studies provide sufficient technical details, such as loading frequency and signal processing algorithms and parameters, to allow replication of the experimental procedure. Standard methods of converting the raw spectral data to the complex OCT signal are well established²², so these steps are often not detailed. However, methods used to convert raw OCE complex data to displacement and wave speed, should be, and often are, detailed thoroughly, including critical parameters such as kernel size and fitting algorithms and the associated mechanical models, for example, Rayleigh or Lamb waves. Recent work focused on complex nonlinear elastic models that incorporate more parameters, such as anisotropy^{51,94,150,168,192,196}, are not widely used. This limits the reproducibility of results and makes comparison of different studies difficult. Furthermore, when considering mechanical properties such as anisotropy, nonlinear elasticity and viscoelasticity, fabricating phantoms that reliably replicate these parameters as in tissue is challenging. Regarding the reproducibility of tissue results, most effort

has focused on technical development, so there are no established clinical databases, and there are relatively few large-scale OCE studies (for example, $N > 50$). However, we expect this to change in the coming years, as several OCE techniques, particularly transverse wave OCE and compression OCE, are reaching technical maturity, and larger-scale studies are emerging^{30,31,33}. It is also worth noting that validation of OCE against more established elasticity techniques, such as ultrasound elastography, magnetic resonance elastography and AFM, should be treated with caution, as the scale dependence of mechanical properties makes such comparisons challenging and potentially misleading³.

We suggest that OCE studies should describe several key parameters to promote the reproducibility and interpretation of OCE results. These minimum reporting standards should include critical information regarding the OCT system specifications, acquisition parameters, OCE hardware, and data processing algorithms and associated parameters. Although no dedicated depositories exist for OCE data, we still suggest uploading raw OCE data – and the related metadata – to open access depositories. Data should be written in the linear-in-wavenumber format for the raw spectral interference data²². Even though many of the data processing algorithms are commonplace due to their adaptation from other fields, for example, seismology and ultrasound elastography, data processing codes are not often shared explicitly with publications, but the authors strongly encourage the community to make these codes available, barring any intellectual property exceptions.

Limitations and optimizations

OCE has undergone considerable development. We describe the results of technical studies focused on assessing the performance of OCE and subsequent techniques for improving the applicability and performance of OCE. These studies can guide which parameters to optimize for specific applications and goals in future OCE studies.

Quantitative estimation of mechanical properties

As OCE matures, quantification of mechanical parameters has come under the proverbial microscope. In the early stages of OCE development, very simplified mechanical models were utilized, based on assumptions such as isotropy, infinite thickness and pure elasticity^{15,16}. Now, the simultaneous detection of multiple parameters for enhanced mechanical contrast is emerging as an important new path for OCE. For example, with compression OCE, the detection of the full strain tensor can account for artefacts, such as voids due to ducts or vessels⁹⁴, and the quantification of the in- and out-of-plane elasticity of the cornea may explain the large scale of discrepancies between elastographic measurements (tens of kPa with OCE) and mechanical testing (a few MPa)¹⁶⁸. Measurement of these more complete and complex mechanical

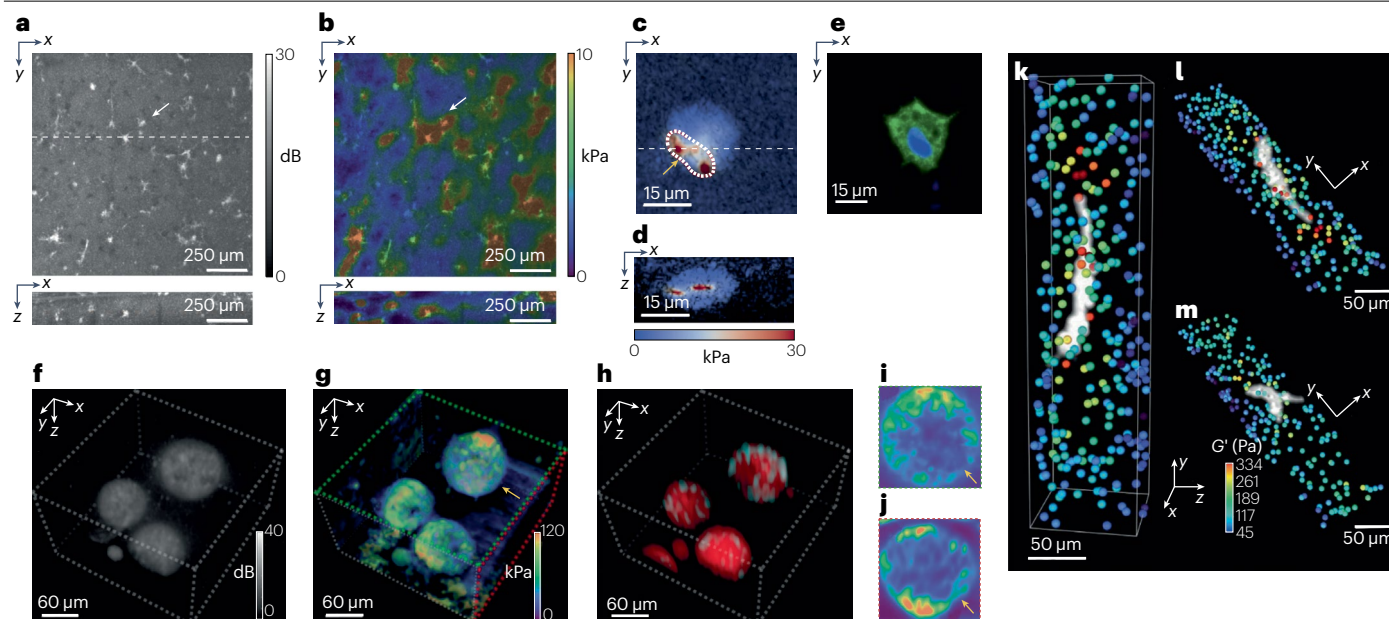


Fig. 6 | OCE in mechanobiology. **a, b**, Optical coherence tomography (OCT) (part **a**) and OCT/quantitative micro-elastography (part **b**) overlay images of cells obtained from compression optical coherence elastography (OCE). Cross-sections in the xy and zx planes are displayed in the top and bottom images, respectively. The white arrows indicate example regions where individual cells have elevated elasticity⁵⁷. **c–e**, Images of a cell acquired using mechano-microscopy in the xy (part **c**) and zx (part **d**) planes. The yellow arrow in part **c** indicates elevated elasticity probably corresponding to the nucleus. Representative confocal fluorescence microscopy of a cell as in parts **c** and **d** with blue and green representing the nucleus and cytoskeleton, respectively (part **e**)⁵⁸. **f–h**, Images of cell spheroids acquired by multimodal mechano-microscopy, that

is, fluorescence imaging and compression OCE: optical coherence microscopy signal-to-noise ratio (part **f**), elasticity (part **g**) and fluorescence labelled with nuclear (cyan) and membrane (red) fluorescent dyes (part **h**). The yellow arrows indicate the same spheroid in parts **i** and **j**. **i, j**, The elasticity of a spheroid over a $120 \times 120 \mu\text{m}$ field of view in the xy (part **i**) and zx (part **j**) planes²³³. **k–m**, Three-dimensional mapping of extracellular matrix stiffness surrounding a cell using photonic-force OCE (part **k**) and projection along z of the stiffness of the cellular microenvironment before (part **l**) and after (part **m**) treatment with an actin polymerization inhibitor⁵⁹. Parts **a** and **b** adapted with permission from ref. 57, OPTICA. Parts **c–e** adapted with permission from ref. 58, OPTICA. Parts **f–j** adapted from ref. 233, CC BY 4.0. Parts **k–m** adapted from ref. 59, CC BY 4.0.

properties comes with several trade-offs. In anisotropic measurements in transverse wave OCE, nonlinearity and the presence of multiple wave modes can confound quantification¹⁶⁸, but specialized models have been demonstrated to account for nonlinearity and pre-stress²⁵⁰. This exciting area of OCE is ripe for further development, and we anticipate notable progress in the near future.

Sources of errors in the quantification of mechanical properties have also been investigated. In the current mechanical model for compression OCE, stress is assumed to be uniaxial; however, factors, including mechanical heterogeneity, uneven surface topology and friction, invalidate this assumption, which can lead to elastogram artefacts. Several approaches are likely to be crucial in overcoming this limitation. Mapping the 3D strain tensor and solving mechanical models that rely on fewer assumptions using FEM is key to overcoming challenges with tissue mechanical heterogeneity. FEM can incorporate spatially varying stiffness and hyperelastic behaviour²⁵¹ and models of anisotropy²⁵². Anisotropic models could also be used in combination with co-registered fibre orientation measurements from polarization-sensitive OCT^{51,253}, which can measure the orientation of ordered structures such as fibres²². Inverse problem-solving approaches, such as FEM or machine learning, can probably be used to estimate 3D stress from the application of specific boundary conditions to measured 2D strain maps²⁵⁴. In addition, better understanding the impact of surface topology²⁴⁸ and friction^{103,104} on elastogram quality can also aid in identifying artefacts

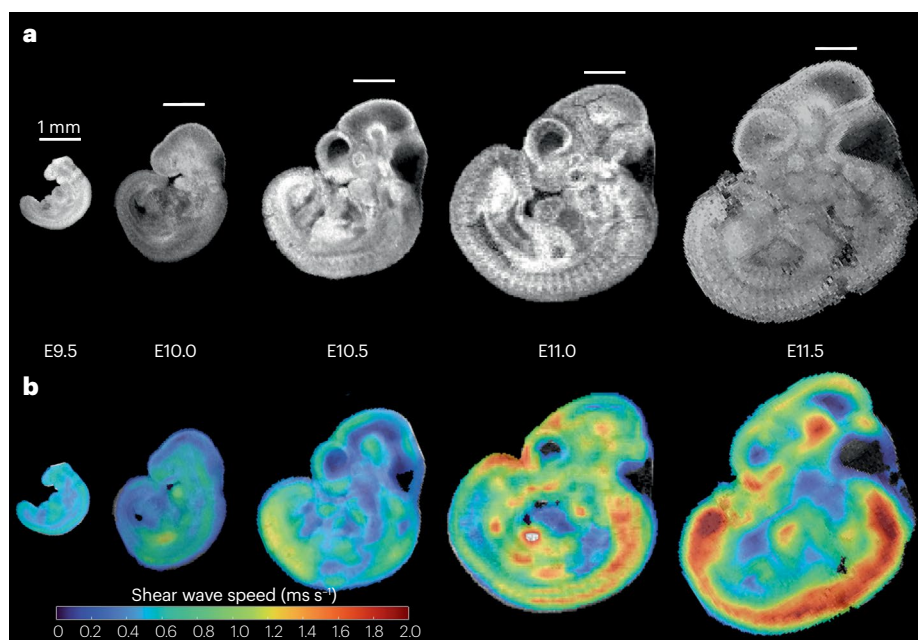
and avoid erroneous interpretation of contrast in the elasticity maps. Combining these approaches will probably allow compression OCE to improve elastogram quality in complex tissues and multicellular models such as spheroids and organoids. In wave-based OCE, the boundary conditions specifically have powerful effects on the accuracy of mechanical quantifications. For example, waves in thin tissues such as the cornea are guided waves that are heavily influenced by the sample thickness^{39,168,169,175}, and boundary conditions such as the presence of a solid–fluid interface can dramatically affect wave propagation¹⁶⁹. As in compression OCE, FEM and analytical methods have both been demonstrated to reduce inaccuracies in wave-based OCE by integrating the effects of boundary conditions^{168–170}. Artefacts can arise due to other effects, such as the near-field effect, which is clearly shown in Fig. 4f–h by the high speeds near the excitation at the corneal apex. However, these regions are usually ignored during data analysis.

Contrast, resolution and sensitivity

Determinants of several key metrics for image quality have been rigorously studied in OCE. These studies often incorporate FEM and models of OCT image formation. An early study of compression OCE elastogram quality used FEM and structured silicone phantoms to show that sample mechanical contrast directly impacts the measured elastogram contrast⁹⁹. The assumption of uniform stress is accurate in low mechanical contrast and layered structures, which results in high

contrast of the elastogram. Moreover, this work predicted a minimum detectable elasticity contrast ratio of 1.002:1 in a noiseless scenario. Following this, a cornerstone study in understanding OCE image formation combined FEM with a linear system model of OCT to demonstrate

how the coupling between sample deformation and the optical measurement directly impacts strain sensitivity⁷⁶. The ideas developed in these studies were subsequently used to investigate spatial resolution and elasticity sensitivity.



c Displacement of the renal tissue at 2.00 ms after excitation

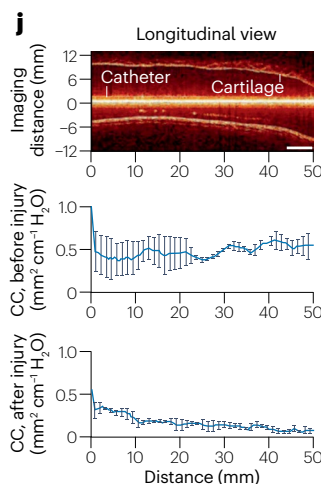
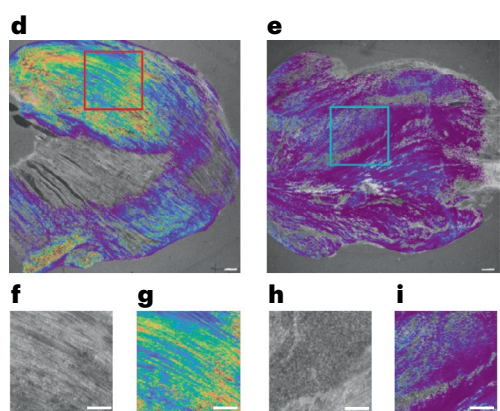
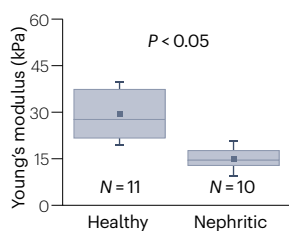
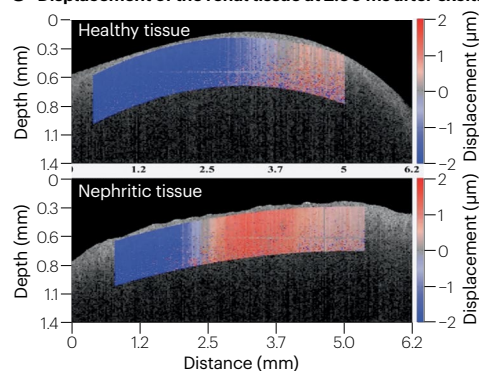


Fig. 7 | Emerging OCE applications. **a,b**, High-resolution mechanical mapping of whole murine embryos with reverberant optical coherence elastography (OCE) showing the optical coherence tomography (OCT) structural image maximum intensity projections (part **a**) and local elastic wave speed (part **b**) in a central sagittal plane of embryos at the denoted developmental stages²³⁴. **c**, OCE measurements, at 2 ms after excitation, showing elastic wave propagation in the typical healthy (upper image) and nephritic murine kidneys (lower image). The plot on the right compares the measured Young's moduli between the two cases where the square dots and whiskers represent the mean and standard deviation values, respectively²³⁵. **d,e**, OCE of skeletal muscle tissue studying muscular dystrophy: OCT and elasticity overlays of ex vivo murine quadriceps before (part **d**) and after (part **e**) the onset of dysferlinopathy. The red square highlights region of interest, including ordered striations in the muscle and the cyan square indicates region of muscle being replaced by soft adipose tissue. **f–i**, The corresponding magnifications of the regions of interest from part **d** (parts **f** and **g**) and part **e** (parts **h** and **i**)²⁴⁰. Scale bars, 500 μm (**d** and **e**) and 250 μm (**f–i**). **j**, OCE of an ex vivo porcine trachea. OCT image in a longitudinal cross-section (top) and the corresponding plots of cross-sectional compliance (CC) before (middle) and after (bottom) a steam inhalation injury²⁴³. Scale bar, 5 mm. E, embryonic day. Parts **a** and **b** adapted with permission from ref. 234, OPTICA. Part **c** reprinted with permission from ref. 235, Wiley. Parts **d–i** adapted with permission from ref. 240, OPTICA. Part **j** adapted with permission from ref. 243, IEEE.

In early OCE studies, mechanical resolution in compression OCE was stated to match the lateral resolution of OCT, and the window length (Δz) therein used for axial strain estimation^{15,67}. However, it has been demonstrated that this is not the case in compression³⁶, transverse wave^{37,39} and axial wave OCE³⁸. The mechanical resolution can be considered as a convolution of the OCT system resolution, the intrinsic mechanical resolution (that is, mechanical feature) and signal processing, so the resolution is often degraded by an order of magnitude or more in OCE as compared with the parent OCT system. System resolution is defined as the combination of OCT and signal processing resolution and is independent of the sample. Feature resolution is defined as the measured resolution of features in the elastogram, such as a transition between a stiffer and softer region in a side-by-side phantom or a stiffer solid tumour within a softer surrounding tissue.

In compression OCE, as the compressive load is applied across the sample surface, local deformation varies due to mechanical heterogeneity. As a result, the resolution of mechanical properties varies throughout the elastogram. Mechanical resolution in compression OCE was investigated by separating it into two aspects: system resolution and feature resolution. Sample deformation was identified as the primary factor influencing feature resolution. Furthermore, it was shown that feature resolution varies along the feature boundaries and is influenced by feature size, geometry and mechanical contrast. Importantly, sample deformation often results in the axial and lateral mechanical resolution being generally at least one order of magnitude coarser than the OCT system resolution in structured phantoms. Practically, this means that the mechanical resolution in compression OCE is $\sim 70\ \mu\text{m}$ if the OCT system resolution is $\sim 5\ \mu\text{m}$ and there is large mechanical contrast. Using a similar approach, elasticity sensitivity has also been studied. Sensitivity is dependent on factors including the OCT SNR, the length of the fit window in signal processing and the extent of temporal averaging, but, importantly, can be lower than 1 kPa (ref. 102). The sensitivity and resolution can be either improved simultaneously through temporal averaging, or improved individually, at the expense of the other. For selected applications, resolution can in fact be traded off to improve sensitivity and optimize elastogram quality.

The sensitivity in transverse wave OCE was studied by measuring the variance in wave speed of repeated measurements in phantoms, and the results showed that a change in wave speed of $<4\%$ could be reliably detected²⁴⁴. Additional studies analysing the spatial resolution in transverse wave OCE showed similar results, where the OCE resolution was much coarser than the OCT resolution^{37,39}. The mechanical feature resolution along the direction of the wave propagation was tightly correlated with the stimulation frequency and spot size; a higher frequency and smaller spot size resulted in a finer mechanical. For example, ACUS excitation at 1 kHz resulted in a spatial pulse width of $\sim 3\ \text{mm}$ and a mechanical resolution of $\sim 1.5\ \text{mm}$. Additional work in this critical study also showed that the pulse width had a strong effect on the mechanical contrast and accuracy of the detection of a stiff inclusion smaller than the wavelength of the mechanical wave. As with compression OCE, there is a trade-off between resolution and SNR. However, Kirby et al.³⁷ showed that the elastogram SNR could be substantially increased with only a minimal loss in resolution by using a kernel-weighted averaging technique into the local speed calculation. Much work in transverse wave OCE is in thin media (for example, the cornea) and additional research showed that the mechanical feature resolution becomes coarser in thicker samples³⁹. The axial (z) mechanical feature resolution is also a critical parameter, and two major techniques have been demonstrated

with the goal of improving this resolution: longitudinal shear waves²⁴⁶ and reverberant OCE¹¹². Although it has been reported that noise-correlation methods based on these techniques²⁵⁵ (for example, reverberant and diffuse shear techniques, and shear wave elastography²⁵⁶) have super-resolution capabilities, rigorous characterization of their performance is still needed.

To understand the effects of excitation localization on mechanical feature resolution and contrast in harmonic OCE, wide and local ARF stimulation was applied in structured tissue-mimicking phantoms³⁸. The results show that increasing the excitation frequency leads to a finer mechanical feature resolution for all types of excitations. Moreover, the resolution was finer and the mechanical contrast was greater with a more localized excitation, although such an excitation for large FOVs would increase the imaging time dramatically as the source would need to be moved as well.

Although these parameters have been investigated in these three OCE techniques, they have not been explored in all the presented methods described in this Primer. Moreover, these parameters have been quantified on different types of phantoms with different numerical definitions and paradigms. Hence, a direct comparison on the full capabilities of each OCE technique is not yet available and is clearly an area that should be actively pursued with activities such as dissemination of identical phantoms between research groups as described in the 'Reproducibility and data deposition' section.

Imaging speed

The development of ultrafast OCT systems has enabled volumetric imaging at over 30 Hz (ref. 22), dramatically reducing OCE acquisition times, as demonstrated with transverse wave OCE^{187,191,247}. Wave-based OCE methods require the acquisition of temporal information, usually over a few milliseconds (equivalent to few hundred A-lines with typical OCT systems), but ultrafast OCT system have enabled new imaging paradigms⁶⁴. Typical OCT systems utilize M-mode imaging methods, which consists in holding the OCT beam stationary for wave-based OCE and results in 2D acquisition times of the order of seconds to tens of seconds, going up to even a few minutes for 3D acquisitions^{16,64}. With ultrafast OCT systems, temporal information can be acquired while scanning the beam, reducing the 2D and 3D acquisition times to a few milliseconds and a few seconds, respectively^{191,247}. Temporal information is not needed for compression OCE, where the two OCT datasets of unloaded and loaded volumes are required (Fig. 2). Recent work even utilized a rapid scanner to acquire data of the two load states on the forwards and backwards scans separately. Thus, volumetric compression OCE required less than 4 s, making results like this crucial for intraoperative applications where speed is a critical factor³⁵. In other cases where speed is not a concern, temporal averaging can substantially improve both the elastogram quality and mechanical sensitivity in compression OCE^{74,102}.

FOV and imaging depth

OCE is positioned as a mesoscale technique, with a larger FOV than nanoscale techniques such as AFM¹¹ but a smaller FOV than ultrasound and magnetic resonance elastography³. The imaged FOV is determined by the underlying OCT imaging system, which is often $\sim 1 \times 1\ \text{cm}$. However, wide-field imaging ($\sim 5 \times 5\ \text{cm}$) by mosaicking has been demonstrated in compression OCE²⁰⁶. On the other end of the scale, OCM-based compression OCE has a much more confined FOV to $\sim 1 \times 1\ \text{mm}$ ⁵⁸, demonstrating the trade-off with FOV and resolution. In transverse wave OCE, the FOV can be predominantly limited by the

mechanical properties of the sample and the frequency of the excitation. In a viscoelastic medium, damping is notably greater at higher frequencies so that FOV is ultimately limited by the propagation distance of the wave^{16,64}. For example, under contact-based excitation, mechanical waves in the skin and cornea above 10 kHz are almost completely damped within a propagation length of 2 mm (refs. 52,171,185), making the effective FOV much smaller than the limit determined by the scanning system (usually $\sim 1 \times 1$ cm). As a solution, multiple excitations²⁵⁷ or mosaicking¹⁸⁵ could expand the FOV, but this is not widely implemented in transverse wave OCE owing to practical limitations with extended imaging time and translation of the sample or OCE system. In axial wave OCE methods, the entire sample can be vibrated such that the FOV is limited by the optical set up, but wide-field excitation can reduce the mechanical resolution and contrast³⁸.

The imaging depth is largely defined by the OCT system, which generally provides sufficient SNR down to 1–2 mm in tissues with typical OCT systems. OCE carries over the imaging depth, so OCE applications are often focused on pathologies with superficial presentations, such as in the eye or skin, or applications where access can be provided with probes, such as in the airways or blood vessels^{15,16}. Although the imaging depth can be limited, mechanical properties can be sensed further down inside the tissue thanks to the mechanical coupling between the deeper and shallower tissue. For example, transverse propagating mechanical waves with very long wavelengths (>5 mm) would be able to sense mechanical properties from deeper than the OCT imaging depth because the penetration depth of the waves, that is, how deep the waves can ‘sense’ mechanical properties, is proportional to their wavelength^{3,16,258}. Similarly, optical palpation with compression OCE has also shown mechanical sensing beyond the OCT imaging depth because the stress on the surface is sensitive to the mechanical properties of the entire thickness of the tissue⁹⁰. However, the stress is more heavily influenced by superficial tissues.

Safety

Although OCT and OCT angiography are FDA approved, the imaging and loading safety of OCE need to be thoroughly investigated. In compression OCE, safety concerns are largely focused on sterility owing to contact with the sample (for example, during open surgery), which has been solved with sterile disposable stress sensors and sheaths^{35,42}. In wave-based OCE methods, safety concerns are instead focused on tissue loading, as localized loading is often repeated hundreds of times in a few seconds, for example, a pulse repetition frequency of ~ 25 Hz (ref. 259) and on laser radiation, as the OCT beam is typically held stationary for a few milliseconds at one location (M-mode). In these instances, laser radiation can be particularly dangerous for sensitive tissues such as the retina, for which special care should be taken. Methods that may be technically attractive due to short and highly localized stimulation, such as focused laser pulse²⁶⁰, are usually not safe. Even though non-contact methods such as a focused laser pulse or ACUS are preferred, contact-based methods have shown no damage to tissues such as the cornea because OCE can detect minuscule motion¹⁸⁴. Rigorous safety characterizations of prominent loading methods, such as ARF, are currently in progress, with both ex and in vivo results showing that ARF may exceed safety limits in many OCE applications. However, damage to tissues, in this case the crystalline lens, was not seen until well over ten times the safety limit^{259,261}. Ultrafast OCE methods are highly attractive because the OCT beam is rapidly scanned, and only a single stimulation is required for a 2D measurement^{187,191,247}. By contrast, laser safety and loading are generally not a concern in compression OCE

Glossary

Elastogram

An image where the contrast is based on mechanical properties of the sample, such as Young's modulus.

Group velocity

The speed at which the wave packet — composed of many frequencies — propagates.

Lamb wave

Dispersive guided elastic waves that propagate in thin plates and can have multiple modes.

Linear elastic

The theory of elasticity that assumes the deformation in a specimen is small, and that the relationship between forces and deformation is linear and independent of time.

Low coherence interferometry

An optical technique using a beam of low-coherence light to measure axial positions from weak back reflections of a sample, sent to an interferometer.

Phase velocity

The speed at which a point of constant phase propagates as a wave propagates, also seen as the velocity of a single-frequency component in a wave packet.

Poroelectric

Materials possessing a liquid and solid porous phase whose interplay influences the mechanical properties of the material.

Strain

The normalized deformation in a material, where the displacement is divided by the original length of the sample.

Stress

The force per unit area applied to a material.

Viscoelastic

Materials such as most tissues whose mechanical properties have both a time-independent elasticity and a time-dependent viscosity.

Voxel

The three-dimensional counterpart to a pixel.

Wave speed

The speed of a wave, which in solid mechanics, is closely related to the square root of shear or Young's modulus of a tissue.

as the OCE beam is continually scanned, and the loading is generally small (<100 Pa) and spread over a large area¹⁵.

Outlook

Elastography has proven its value in the clinic at the organ scale^{3,5}, and new insights in mechanobiology are being revealed with nanoscale techniques^{9,225}. OCE is positioned between these two scales, and its unique combination of features has led to its steady growth; there has been an average annual growth in the number of publications and citations of 24% and 45%, respectively, since the seminal OCE publication in 1998 (based on a Web of Science topic search for ‘optical coherence elastography’ on 6 October 2024).

OCE is just now reaching technical maturity for large-scale clinical studies, and we expect the near future to cement the clinical utility of OCE, like its ultrasound⁴ and magnetic resonance⁵ predecessors. Towards clinical translation, compression OCE³⁵, axially transient OCE¹¹⁹ and transverse wave OCE^{53,189} have all shown excellent in vivo repeatability^{35,119}. Following these repeatability studies, OCE clinical investigations have shown great promise for the detection of keratoconus^{30–32} and breast cancer^{33,35} in live patients. However,

large-scale studies are required for the regulatory approval of OCE and assessing its impact in these and other medical areas, and OCE commercialization will undoubtedly have a critical role in expanding OCE availability in the clinic.

One of the most critical areas for ongoing and future OCE development is the form factor; most OCE systems are benchtop focused, which is not conducive to clinical imaging in patients. To this end, handheld probes^{35,40,41}, mobile systems^{33,53} and integration into existing clinical examination hardware³⁰ have been demonstrated, but challenges still remain. For example, handheld probes for wave-based imaging have not been demonstrated but they would enable imaging of patient skin sites assessed during systemic sclerosis examinations that are not accessible with benchtop form factors²²⁴. Here, ultrafast techniques show great promise for minimizing the effects of hand and patient motion.

Providing real-time assessments would also improve the clinical viability of OCE, and artificial intelligence (AI) shows promise for accelerating OCE data processing. AI methods in OCE data processing are still nascent, but show excellent preliminary results^{61,164,165}. AI has also shown significantly improved disease classification performance with OCE^{33,235}, and we expect AI to have a substantially greater part in the future of OCE.

In addition to mechanical imaging, other OCT functional extensions can be easily integrated, such as polarization-sensitive and angiographic imaging to investigate tissue integrity from multiple aspects^{51,253}. This multifaceted view of tissue health may be a powerful tool for predicting disease progression, evaluating therapy response and planning personalized interventions.

Finally, there is a large focus on developing highly accurate and sensitive OCE techniques, including hardware, data processing algorithms and mechanical models to tease out minute mechanical contrast, especially for early disease detection. There is also a need for techniques and associated models of more complex mechanical properties that truly reflect the complex biomechanical nature of biological samples, and tremendous progress has been made to assess tissue nonlinearity^{157,250}, viscoelasticity^{26,167,170,171,250} and mechanical anisotropy^{51,94,150,168,196}.

Currently, there are no commercially available OCE systems but, to the best of our knowledge, at least two startup companies are working on developing one: ElastEye Inc. in the USA and OncoRes Medical in Australia. We envision the first commercial applications to be in the areas of ocular or skin biomechanics and tumour margin detection in breast-conserving surgery.

The outlook for OCE is very bright, as it is rapidly progressing and is showing its capabilities for accurate, robust and high-resolution mechanical imaging in ever-broadening applications.

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Author contributions

Introduction (M.S., M.S.H., B.F.K. and K.V.L.); Experimentation (M.S. and M.S.H.); Results (M.S. and M.S.H.); Applications (M.S. and M.S.H.); Reproducibility and data deposition (M.S. and M.S.H.); Limitations and optimizations (M.S., M.S.H., B.F.K. and K.V.L.); Outlook (M.S., M.S.H., B.F.K. and K.V.L.).

Competing interests

M.S. and K.V.L. are inventors of OCE patents owned by the University of Houston and have a financial interest in an organization that may gain or lose financially through this publication. B.F.K. is co-founder of OncoRes Medical, a company in which he has a financial interest and from which he has received research funding. M.S.H. declares no competing interests.

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